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CONTENTS

The agar plate spore germination method for testing fungicides — M. L. Gattani 113

Electron microscopy and seed and pollen transmission of rod-shaped particles associated with the false stripe virus disease of barley
A. Herbert Gold, Coit A. Suneson, Byron R. Houston, and John W. Oswald 115

Ringspot-like virosis of rhubarb — John W. Yale, Jr. and Edward K. Vaughan 118

Two recently isolated strains of curly top virus — N. J. Giddings 123

Some studies of curly top on potatoes — N. J. Giddings 125

Dieback and canker of camellias caused by Glomerella cingulata
L. W. Baxter and A. G. Plakidas 129

A new fruit rot of peach — Norman L. Horn and P. L. Hawthorne 134

Relation of Stagonospora, Rhizoctonia, and associated fungi to crown rot of alfalfa
Donald C. Erwin 137

Use of actidione in culture of plant pathogenic bacteria (A phytopathological note)
W. F. Jeffers 144

Some factors affecting development and control of adaxial crack stem of celery
J. B. Kendrick, Jr., R. T. Wedding, John T. Middleton, and Bernarr J. Hall 145

Plugging of vessels by tyloses and gums in wilting oaks
B. Esther Struckmeyer, C. H. Beckman, J. E. Kuntz, and A. J. Riker 148

Production of vascular browning by metabolites from several pathogens
N. N. Winstead and J. C. Walker 153

Toxic metabolites of the pathogen in relation to Fusarium resistance
N. N. Winstead and J. C. Walker 159

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THE AGAR PLATE SPORE GERMINATION METHOD FOR TESTING FUNGICIDES [1]

M. L. Gattani

SUMMARY

The method described in this paper for evaluating fungicides utilizes an agar surface, instead of the conventional water drop, for determining spore germination; this reduces the day to day variations in spore germination. The method requires a minimum of equipment and has been found suitable for testing compounds insoluble in water but soluble in liquids miscible with water.

This paper describes a spore germination method of evaluating fungicides that has advantages over the slide method recommended by the American Phytopathological Society's Committee on the Standardization of Fungicidal Tests.[2,3] It requires a minimum of equipment; it is suitable for testing compounds insoluble in water but soluble in liquids miscible in water.

In the slide germination method, the provision for stabilizing test drops on the slides presents considerable difficulty and does not appear to be infallible. Drops containing spores tend to flatten or spread, frequently flowing together or off the slide during the 12–14 hr. period in the moist chambers. Sometimes, depending upon the nature of fungicides, the drops tend to round off as flattened spheres. Naturally the loss of drops decreases the efficiency of the test. With certain types of fungicides, spores in the drops tend either to aggregate in the center or to move towards the periphery of the water drop. This has resulted in marked differences in the germination test studies, often making it impossible to reproduce the results from day to day with the same materials under identical conditions.

PROCEDURE.—It was essential to use chemically clean glassware for these studies. To minimize chances of partial inactivation of chemicals which react with agar, pyridine purified agar is recommended for the tests. As that was not available to the author, ordinary agar was used. For those fungicides which are soluble in water, dilu'ions containing various p.p.m. by weight of the fungicide were prepared in test tubes. For the fungicides which are insoluble in water but the active constituents of which are readily soluble in alcohol or other solvents miscible in water, 0.1–0.5 gm. of the fungicide was first dissolved in a minimum quantity of alcohol, usually from 2.0 ml. to 5.0 ml. Distilled water was then added and the volume increased to 100 ml. Dilutions of known strength were made from the stock suspension.

Meanwhile, Erlenmyer flasks each containing 99 ml. of 1 per cent sterile water agar at 43° C. were set up. One ml. of the dilutions in the test tubes was added to the agar in the flask, which was shaken vigorously to obtain a uniform distribution. Twenty-five ml. aliquots of the agar were immediately poured in petri dishes and cooled.

FUNGI USED IN THE BIOASSAY.—*Alternaria solani* (Ell. and Mart.) Jones and Grout, *Aspergillus niger* van Tieghem, and *Rhizopus nigricans* Ehrenberg, were cultured on petri plates of potato-dextrose agar, as it is easier to get the spore inoculum free from agar by light scraping from plates than from tubes. As recommended,[2] 7-day-old cultures of the fungi were used.

DENSITY OF SPORE SUSPENSION.—It is known that percentage germination varies with the fungicidal dose per spore and that there is a direct relation between the log LD 50 and the density of spore suspension. The Committee on the Standardization of Fungicidal Tests has recommended that the number of spores per low power field (15 × ocular, 16 mm. objective) should be about 35. It was observed that there is no significant change in the LD 50 value if the number of spores per low power field is from 35 to 50 on the agar surface but if the number exceeds 100 there is a slight change in the LD 50 value. The following procedure was used to obtain about 35 to 50 spores per low power field. From the fungal cultures growing in petri plates small quantities of spore material were removed by light scraping with a sterile wire loop, care being exercised not to take any nutrient agar. This was then transferred gently to the petri plate. The blunt end of a test tube (diameter 0.5 in.) held at an angle of 45° was pressed against the inoculum streaked on the plate, care being taken not to cut the agar. In time, the large number of spores which at the beginning were clustered in the center was so reduced that widely-spaced individual spores were obtained. Meanwhile, the petri plate was examined under the low power field and the number of spores counted at 4 or 5 places. If the number per low power field was less than the number required, the process was repeated by taking more inoculum on the needle loop. It was usually advantageous to streak only half the petri plate with 1 fungus species. The other half of the petri plate was then streaked with the other fungus species to be tested. The petri plates were kept at 21–23° for 18–20 hours.

[1] Accepted for publication October 26, 1953.

The author conducted this work while on deputation to the Government of Rajasthan as Plant Pathologist. Present address: Division of Mycology, Indian Agricultural Research Institute, New Delhi, India.

[2] American Phytopathological Society, Committee on the Standardization of Fungicidal Tests. 1943. The slide method of evaluating protective fungicides. Phytopathology 33: 627-632.

[3] American Phytopathological Society, Committee on the Standardization of Fungicidal Tests. 1947. Test tube dilution technique for use with the slide germination method of evaluating protectant fungicides. Phytopathology 37: 354-356.

TABLE 1.—*Percentage inhibition of spores of Alternaria solani, Aspergillus niger, and Rhizopus nigricans by copper sulphate and Gattosan as tested by the agar plate method*

Fungicide	Fungus	Replication	Percentage inhibition of spores Parts per million fungicide					
			50	37.5	25	17.5	10	5
Copper sulphate	A. solani	1	100	87	50	31	15	5
		2	100	87	48	30	13	4
		3	100	84	46	28	12	5
	A. niger	1	97	80	41	22	12	9
		2	98	79	40	21	13	7
		3	98	80	39	25	15	8
	R. nigricans	1	100	100	87	40	15	5
		2	100	100	85	42	17	2
		3	100	100	84	43	17	3
Gattosan	A. solani	1	100	100	97	57	30	5
		2	100	100	97	54	31	3
		3	100	100	100	53	28	2
	A. niger	1	100	100	87	50	22	8
		2	100	100	85	47	27	7
		3	100	100	86	46	25	5
	R. nigricans	1	100	100	97	67	35	10
		2	100	100	98	68	36	8
		3	100	100	100	71	39	6

EXAMINATION FOR GERMINATION.—The procedure outlined by the American Phytopathological Society's Committee on the Standardization of Fungicidal Tests was followed in examining the germination of spores and recording the results. However, the number of spores examined was far greater than the number recommended by the Committee on the Standardization of Fungicidal Tests. In concentrations where the germination was less than 10 per cent, about 500 spores were examined. When the percentage germination was more than 10, about 3,000 spores were counted before deriving the percentage germination figures. This was rendered easy because of the large number of spores on the continuous agar surface. Both LD 50 and LD 95 values were determined as the latter was more practical for comparison of fungicides having curves of a convex, sigmoid, or irregular type and dissimilar slope.

STANDARD FUNGICIDE.—Use of standard Bordeaux mixture has been recommended for general comparisons.[2] Young and Cooper[4] used copper sulphate as the standard fungicide. For the sake of simplicity the author used copper sulphate as a standard.

DETERMINATION OF FUNGICIDAL ACTIVITY.—Water-agar petri plates containing different amounts of fungicide were streaked with fungus spores. The entire petri plate was used for streaking 1 fungus for a particular concentration of the fungicide. After incubation for a limited time, the petri plate was tilted and washed with 5 ml. of water from a wash bottle. The wash water was collected and centrifuged at 3000 r.p.m. for 20 seconds. After decantation another 5 ml. of the spore wash from the second petri plate was added and the process repeated with the third and fourth plate, to replenish the loss of spores in washings. After final washing with distilled water the spores were centrifuged and decanted. The fungicidal action was arbitrarily recorded as having stopped with the final washing. Finally the spores were streaked on water-agar plates in which no fungicide had been incorporated and their germination determined.

One of the chief advantages of the agar-plate germination method is its easy maneuverability and excellent replicability. The writer tested the inherent toxicity of 5 fungicides at different times by ascertaining the percentage inhibition of germination of spores of 3 fungi in different concentrations of the fungicide and in each instance obtained significant reproducible results. Table 1 gives the results of tests, repeated 3 times, with copper sulphate and Gattosan,[5] an organic mercurial seed disinfectant, the active constituent of which is soluble in alcohol. In controls the spores of *Alternaria solani, Aspergillus niger* and *Rhizopus nigricans* gave germination percentages of 95, 70 and 72 per cent respectively. Data for all species were adjusted on the basis of viable spores in the controls by dividing the observed germination by the percentage germination of the controls.

DISCUSSION.—Loegering,[6] studying the spore germination of *Puccinia graminis tritici*, showed that the differences in germination percentages were less when he used an agar surface as the germinating medium than when water was so used. In the present studies,

[4] Young, H. C. and W. H. Cooper. 1917. A method for determining the fungicidal coefficient of lime sulphur and other common fungicides. Michigan Acad. Sc. Rept. 19: 221-236.

[5] Gattosan is produced by Asia Chemicals Ltd., New Delhi, India. Composition not yet released.

[6] Loegering, W. Q. 1941. A satisfactory medium for germination of urediospores of Puccinia graminis tritici. Phytopathology 31: 952-953.

wherein different amounts of fungicides were incorporated into agar and the germination of spores studied on the agar surface, the germination tests conducted at different times showed a high degree of reproducibility. This is probably due to the elimination of water drops as a germinating medium.

The procedure suggested in this paper has been found suitable for testing water soluble fungicides, fungicides soluble in alcohol or other solvents miscible in water or fungicides which can be brought into a state of suspension in water. It was observed that the small amount of alcohol used in making the solution was reduced in the final dilutions to such an extent that it caused no significant deviation in the germination of spores. The procedure simplifies the testing of those fungicides whose active ingredients, though insoluble in water are soluble in alcohol. In view of this fact, as well as the easy maneuverability and excellent replicability of this procedure, the method might serve as a useful tool for the assay of the inherent toxicity of a certain class of fungicides.

SECTION OF PLANT PATHOLOGY
DEPARTMENT OF AGRICULTURE
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BHARATPUR, INDIA

ELECTRON MICROSCOPY AND SEED AND POLLEN TRANSMISSION OF ROD-SHAPED PARTICLES ASSOCIATED WITH THE FALSE STRIPE VIRUS DISEASE OF BARLEY[1]

A. Herbert Gold, Coit A. Suneson, Byron R. Houston, and John W. Oswald

SUMMARY

Barley false stripe has been found in California. The disease has been found to be associated with the presence of rod-shaped particles approximately 30 mμ in width, and with an average length of 130 mμ. These particles occur in high concentration, and are found in leaves, embryos, endosperm, pollen, and unfertilized pistils. Pollen transmission of the disease was suggested by the presence of the rods in seed produced from healthy pistils pollinated by pollen from diseased plants. Seed from diseased pollen and healthy pistils produced a small percentage of diseased seedlings. Electron microscopy of the particles in preparations from individual seeds or parts thereof was found to be practical and convenient.

Barley false stripe has been recognized as a virus disease for only a short time (2, 3), and has not previously been reported in California. The disease was found to be seed transmitted (2), with up to 50 per cent of the seedlings showing symptoms. More recently, (4), it was shown that up to 90 per cent of seedlings from seeds of diseased plants may carry the virus, but that symptom expression occurs in a much smaller proportion. This high percentage of seed transmission and masking of symptoms obviously presents special problems to the breeder of barley. The purpose of this paper is to report electron microscopy of particles which we have found in various organs of diseased plants inoculated from barley false stripe material collected in California.

Sources of the virus.—The virus used came from 3 sources. The variety Hanna, C.I. No. 8060, seed of which was received from Aberdeen, Idaho, in 1951 for routine testing, expressed false stripe in 1 of 2 field plantings observed in 1952. Natural infection was observed in a commercial planting of California Mariout barley on the University Farm, Davis, California, in the spring of 1952. The preliminary plant tissue and seed studies reported herein were with this seed stock or mechanical transfers therefrom to other barley or wheat plants.

The other source of false stripe virus was plants originally selected from the Club Mariout variety in 1947 "because of partial sterility and somewhat shorter straw" (factors now known to be associated with false stripe). Not until 1951 was false stripe actually recognized in this stock. Since then, field expression with chlorotic markings on the foliage has generally exceeded 50 per cent. Successive efforts to rogue plants expressing symptoms and to select only the most vigorous progeny have not produced any virus-free seed stocks. All reported studies with pollen, pistils, or immature seed involve this stock or hybrids derived from it.

Hybrids between male sterile barley, a tool long used in California for study of floral infection (8), and diseased Club Mariout were grown in 1951, 1952, and 1953. In addition, the male sterile character has been back-crossed into several standard varieties. Hence in 1953, it was possible to cross reciprocally to healthy or diseased male-sterile plants. Because of the nature of their open glumes it was possible to pollinate them by shaking pollen over the open florets, with minimum likelihood of contamination by contacts other than the pollen itself.

Rod-shaped particles in leaf tissue of diseased plants.—Samples of leaf tissue from healthy and experimentally infected Club Mariout barley plants were prepared for electron microscopy by grinding in a mortar with a little water, then making up the homogenate to 100-fold dilution of the original tissue with addi-

[1] Accepted for publication October 27, 1953.

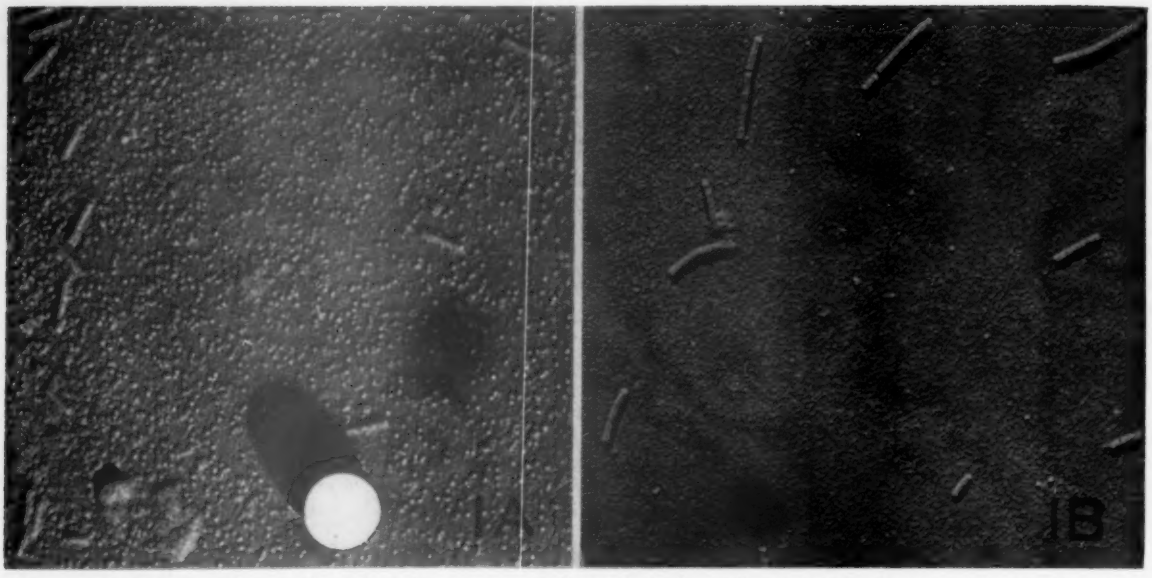

FIG. 1. A) Electron micrograph of leaf homogenate of di ased barley, diluted 1:100, showing rod-shaped particles. The polystyrene ball is 0.26μ in diameter. B) Same prepiration as 1A after 2 cycles of ultracentrifugation, showing aggregation and breakage.

tional water. To 1 ml. aliquots of the diluted homogenates were added 0.1 ml. of polystyrene latex containing 1.6×10^{11} particles per ml. The mixture was sprayed on collodion-coated electron microscope screen and shadowed with palladium. Diseased leaves were found to contain numerous short, thick, rod-like particles. (Fig. 1A) These were absent in preparations from healthy leaves. The round particles in Fig. 1A were also seen in healthy preparations. Particle counts from several samples of leaf tissue indicated 10^{12}–10^{13} rod-shaped particles per gm. of fresh leaf tissue.

Leaf samples of several other varieties of barley and of wheat which had been experimentally infected with barley false stripe, were also examined. In each case, presence of the rods was associated with the disease.

Several attempts were made to separate and concentrate the rods from the host material. The cleanest preparations resulted by use of I. R. Schneider's (7) chloroform-water emulsion method, but most of the rods were lost, and those that remained were mostly joined end-to-end into indefinitely long strings. The most quantitative separation was achieved by a series of cycles of centrifugation at 5000 and 26000 $\times$ gravity. The pellet at 5000 $\times$ gravity was discarded; that at 26000 gravity was resuspended in 0.1 M ammonium acetate, pH 6.8. Even after 2 cycles of such centrifugation, the preparation was quite impure (figure 1B). In addition, there was evidence of breaking and end-to-end aggregation. Nevertheless, the preparations were found to be infective. If centrifuged at higher speeds than 26000 $\times$ gravity, the rods were increasingly difficult to resuspend in buffer.

Because of breakage and aggregation in semi-purified preparations, particle measurements were made only from electron micrographs of crude preparations. Calibration was based on the polystyrene latex spheres which are 260 mμ in diameter. One hundred and 40 rod-shaped particles were measured. They were found to be about 30 mμ wide, with an average length of 130.7 mμ, a standard deviation of 23.6 mμ, and the standard error of the mean length was 1.99 mμ. As indicated by Fig. 2, the mean length falls close to the mode.

Rod-shaped particles in seed from diseased plants.— Seeds of a number of individual diseased Club Mariout barley plants were planted and 50 per cent to 100 per cent of these seedlings showed symptoms.

Preparations from each of 30 seeds from these seed lots were examined under the electron microscope to determine whether the rod-shaped particles could be observed in seeds, and if so, whether their presence was correlated with symptom expression in the seedlings. Fifteen seeds were from a lot showing 50 per cent symptom expression in the seedlings, and there were 5 seeds each from 3 other lots, one of which had shown 100 per cent infection. The adhering palea and lemma were peeled off of each seed, and the embryo dissected from the endosperm. Five seeds from a healthy source were used as controls. It was found that none of the healthy seeds contained the rod-shaped particles, but all of the 30 seeds from diseased plants contained the particles. The concentration of particles in both embryo and endosperm was approximately as high as in leaf tissue. Thus if the particles prove to be the causative agent for the disease, one would have to postulate inactivation during storage to account for healthy seedlings from a diseased plant.

Four seeds from diseased Sonora wheat were also examined. Only 2 of these contained the rods, and the

concentration was much lower in wheat seeds than in barley.

Rod-shaped particles in floral organs.—The particles were then sought and found in preparation from unfertilized pistils, fertilized but immature pistils, anthers, and pollen from diseased barley plants. Healthy controls were used throughout. The rods were found in every preparation from diseased tissue, and never from healthy preparations.

Pollen transmission of rod-shaped particles.—Because the rod-shaped particles were found in pollen from diseased plants, the possibility of pollen transmission was explored. Comparable healthy plants were pollinated with pollen from diseased and healthy plants. Diseased plants were similarly pollinated. When the resulting seed had reached the soft dough stage, they were examined as described above. The upper half of each spike was used for examination. The lower half of the spike was left on the plant so the seeds could mature for later planting tests. Four individual seeds were examined for each treatment, except that 10 seeds were examined which were derived from diseased pollen and healthy pistils. Rod-shaped particles were found in all seeds of all plants except those of healthy plants pollinated with pollen from heathy plants. In some of the seeds produced from healthy pistils and diseased pollen, the concentration of the rod-shaped particles seemed to be considerably lower than in the seeds from infected pistils. In other seeds from infected pollen, the concentration of rods seemed just as high as that from infected pistils. Matured seeds from the lower halves of the spikes were germinated and grown for determination of symptom expression. Approximately 10 per cent of the seedlings from diseased pollen and healthy pistils showed symptoms. The apparent low percentage of pollen transmission could possibly result from the small amount of virus reaching the pistil and low build up before seed ripening.

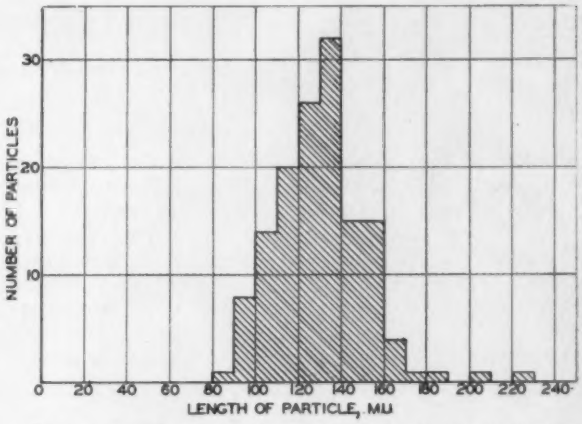

FIG. 2. Length distribution of particles found in barley false stripe tissue.

Pollen transmission in legumes has been suggested by Nelson (5), and Reddick (6). This is the first demonstration of pollen transmission of a virus in the grass family to our knowledge, and it is the first case where seed and pollen transmission have been associated with the presence of a distinctively shaped particle.

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LITERATURE CITED

1. ESLICK, R. F. 1953. Yield reduction in Glacier barley associated with a virus infection. U. S. Dept. Agr. Pl. Dis. Rptr. **37**: 290-291.
2. MCKINNEY, H. H. 1951. A seed-borne virus causing false stripe symptoms in barley. U. S. Dept. Agr. Pl. Dis. Rptr. **35**: 48.
3. MCKINNEY, H. H. 1951. A seed-borne virus causing false stripe in barley. Phytopathology **41**: 563-564.
4. MCKINNEY, H. H. 1953. New evidence on virus diseases in barley. U. S. Dept. Agr. Pl. Dis. Rptr. **37**: 292-295.
5. NELSON, R. 1932. Investigations in the mosaic disease of bean. Michigan State College Agr. Expt. Sta. Tech. Bul. **118**: 1-71.
6. REDDICK, D. AND VERN B. STEWART. 1918. Varieties of beans susceptible to mosaic. Phytopathology **8**: 530-534.
7. SCHNEIDER, I. R. 1953. Solution of tobacco mosaic virus in the aqueous phase of a chloroform-water emulsion and application of this phenomenon in virus assay. Science **117**: 30-31.
8. SUNESON, C. A. AND B. R. HOUSTON. 1942. Male-sterile barley for study of floral infection. Phytopathology **32**: 431-432.

RINGSPOT-LIKE VIROSIS OF RHUBARB [1]

John W. Yale, Jr. and Edward K. Vaughan

SUMMARY

Virus-like symptoms, chlorotic spots, and rings as well as necrotic stippling and rings, were observed in the leaves of rhubarb (*Rheum rhaponticum* L.) in the Willamette Valley of Oregon. The virus was transmitted to healthy rhubarb, Bountiful bean, broad bean, buckwheat, cucumber, curly dock, spinach, Sweet William and zinnia by triturating infected leaf tissue in 0.1 M Na_2HPO_4 and rubbing the extract on leaves dusted with carborundum. Symptoms produced on Bountiful bean and broad bean were comparable with those produced on these hosts by known strains of tomato ringspot virus from cucumber. The symptoms on Sweet William were sufficiently specific for ringspot to place this virus provisionally in the genus *Annulus*. Inoculation tests showed that this virus occurs in naturally infected curly dock in the field.

FIELD OBSERVATIONS.—Several symptom patterns suggestive of virus infection have been observed in rhubarb (*Rheum rhaponticum* L.) in the Willamette Valley of Oregon. Chlorotic spots and rings on the leaves were observed most frequently (Figure 1, A and B); necrotic stippling and rings (Figure 1, C and D) were observed less frequently. Usually only a few leaves showed symptoms on a plant at one time. Symptoms were conspicuous in spring and early summer, becoming less apparent and nearly absent by midsummer. It was estimated that 75 per cent of the plants in 1 field and 60 per cent in another showed symptoms suggestive of virus infection. Neither the size nor the vigor of the plants growing in the field seemed to be reduced by the disease. However, when plants exhibiting the necrotic symptoms were transplanted to pots in the greenhouse, they grew slowly and eventually died. Plants showing chlorotic symptoms remained alive and apparently grew as well as symptomless controls.

Many curly dock weeds (*Rumex crispus* L.) growing in and around the rhubarb fields had leaves with conspicuous mottles and chlorotic rings in their foliage. Leaves were rumpled, narrowed, stunted, and often very red or yellow (Figure 2, B).

EFFECT OF TEMPERATURE.—Typical rhubarb and curly dock plants were transplanted to pots in the greenhouse as sources of inoculum for transmission studies. The old leaves of the rhubarb plants were removed and new ones allowed to grow. In the warm greenhouse all new leaves remained symptomless. When the plants were transferred to glass chambers maintained at 55° and 65° F., symptoms began to reappear on the leaves. Some of the plants collected for chlorotic symptoms in the field now developed a few necrotic rings in the leaves. Vein clearing and mottling were expressed by plants which had not shown these symptoms in the field. Plants with severe necrotic symptoms began to degenerate rapidly at the lowered temperatures. It was apparent that the same plant could develop various symptoms from time to time and that the only recognizable differences between infected plants seemed to be in the severity of symptoms produced.

TRANSMISSION.—Virus transmission was affected by triturating a piece of infected leaf tissue, approximately 2 in. square, in 5 ml. 0.1 M Na_2HPO_4 and rubbing the extract on leaves dusted with carborundum. The leaves were rinsed with water after inoculation.

In the following list of results of transmission studies, the numerator indicates the number of plants infected and the denominator the number inoculated; plants named in capital letters represent transmission from rhubarb; plants named in lower case represent subinoculations from the primary host: CUCUMBER 10/46; Bountiful bean 8/10; spinach 8/8; broad bean 2/8; BOUNTIFUL BEAN 23/36; cucumber 6/10; SPINACH 21/42; buckwheat 17/29; rhubarb 3/10; BUCKWHEAT 3/60; spinach 4/30; CURLY DOCK 3/29; rhubarb 4/10; BROAD BEAN 4/9; SWEET WILLIAM 2/6; ZINNIA 7/19. In addition to tests with the above hosts, attempts were made to transmit the virus to radish, turnip, burley tobacco, pepper, tomato, beet, and nasturtium. There was evidence of transmission to burley tobacco and radish, but further work would be necessary to determine their susceptibility.

Results indicated that the infectivity of the extracts was extremely variable, and in general, the percentage of transmissions was low. During the winter when the growth of test plants was slow, it was difficult to obtain conclusive evidence of virus transmission. The presence of 0.1 M Na_2HPO_4 while triturating infected leaf tissue, increased the infectivity of the extracts. The buffer was less effective when added to leaf juice after trituration.

RELATIONSHIP BETWEEN VIRUS IN RHUBARB AND IN CURLY DOCK.—When the virus was transmitted from naturally infected rhubarb to curly dock seedlings, a pale green mottle accompanied by chlorotic ringspots and leaf rumpling appeared. These symptoms were identical with those observed on curly dock plants in the field. Inoculations from naturally infected curly dock to healthy curly dock and rhubarb seedlings pro-

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The photographs were made by Mr. H. H. Millsap, Oregon Agricultural Experiment Station, Corvallis, Oregon.

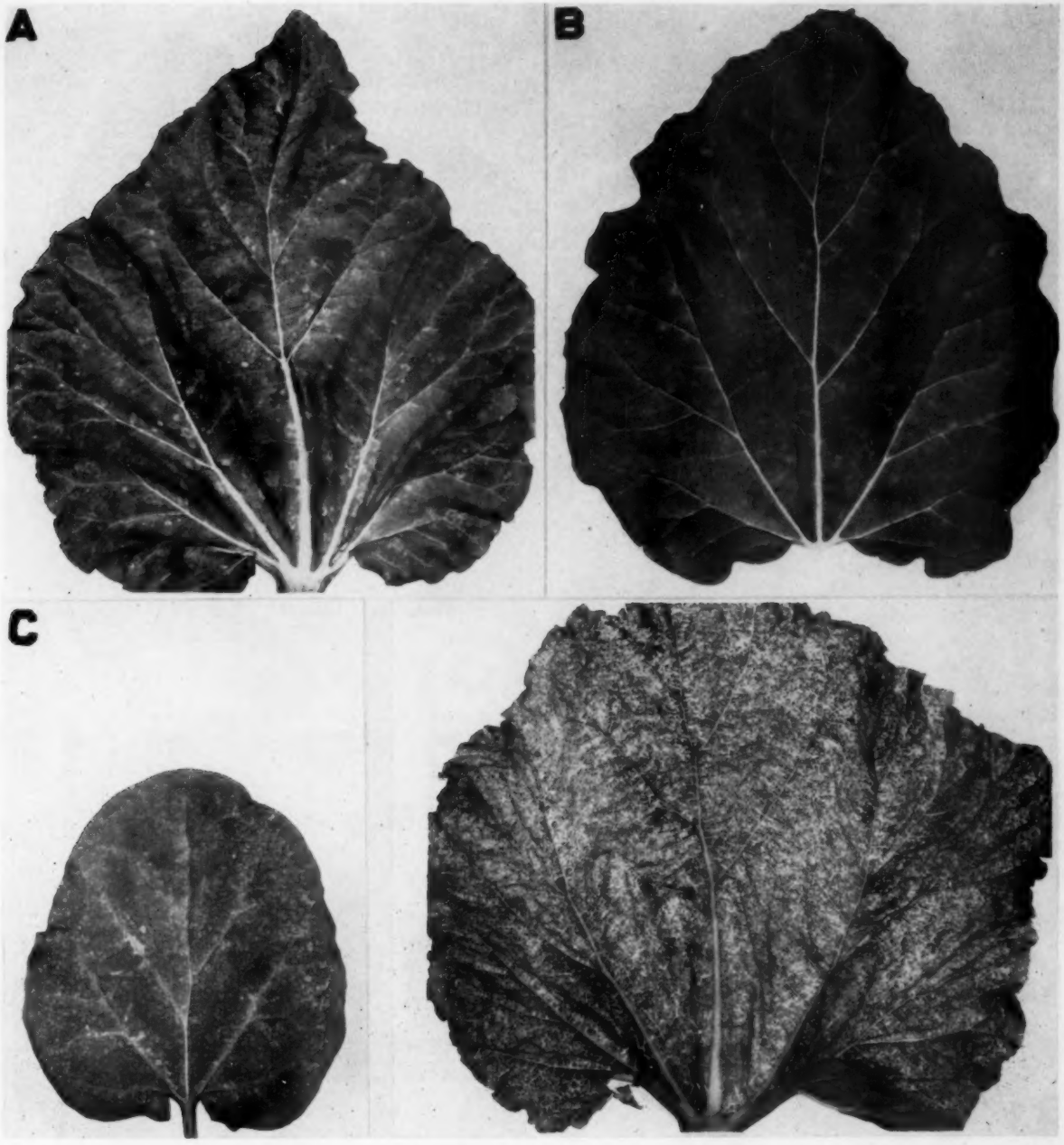

FIG. 1. Virus symptoms in rhubarb. A) Chlorotic rings. B) Chlorotic spotting. C) Early stage of necrotic stippling and rings. D) Late stage of necrotic stippling.

duced the pale green mottle and chlorotic ringspots in curly dock and the characteristic chlorotic spots in rhubarb leaves. These results indicated that the same virus was present in both naturally infected rhubarb and curly dock.

MILD AND SEVERE SOURCES OF VIRUS IN VARIOUS HOSTS. —Rhubarb plants with 2 general types of symptom expression were employed as source plants for making transmissions. One type had either chlorotic or very mild necrotic symptoms, whereas the other type had severe necrotic symptoms and showed a gradual degeneration in growth. The former type will be referred to as the mild source, the latter the severe source. Inoculum from these 2 sources often produced identical symptoms when transmitted to other hosts. Both the mild and severe rhubarb sources inoculated to cucumber produced circular, chlorotic local lesions in the cotyledons. The lesions became necrotic on the undersurface of the cotyledons and increased in size as the cotyledon aged. The true leaves developed chlorotic

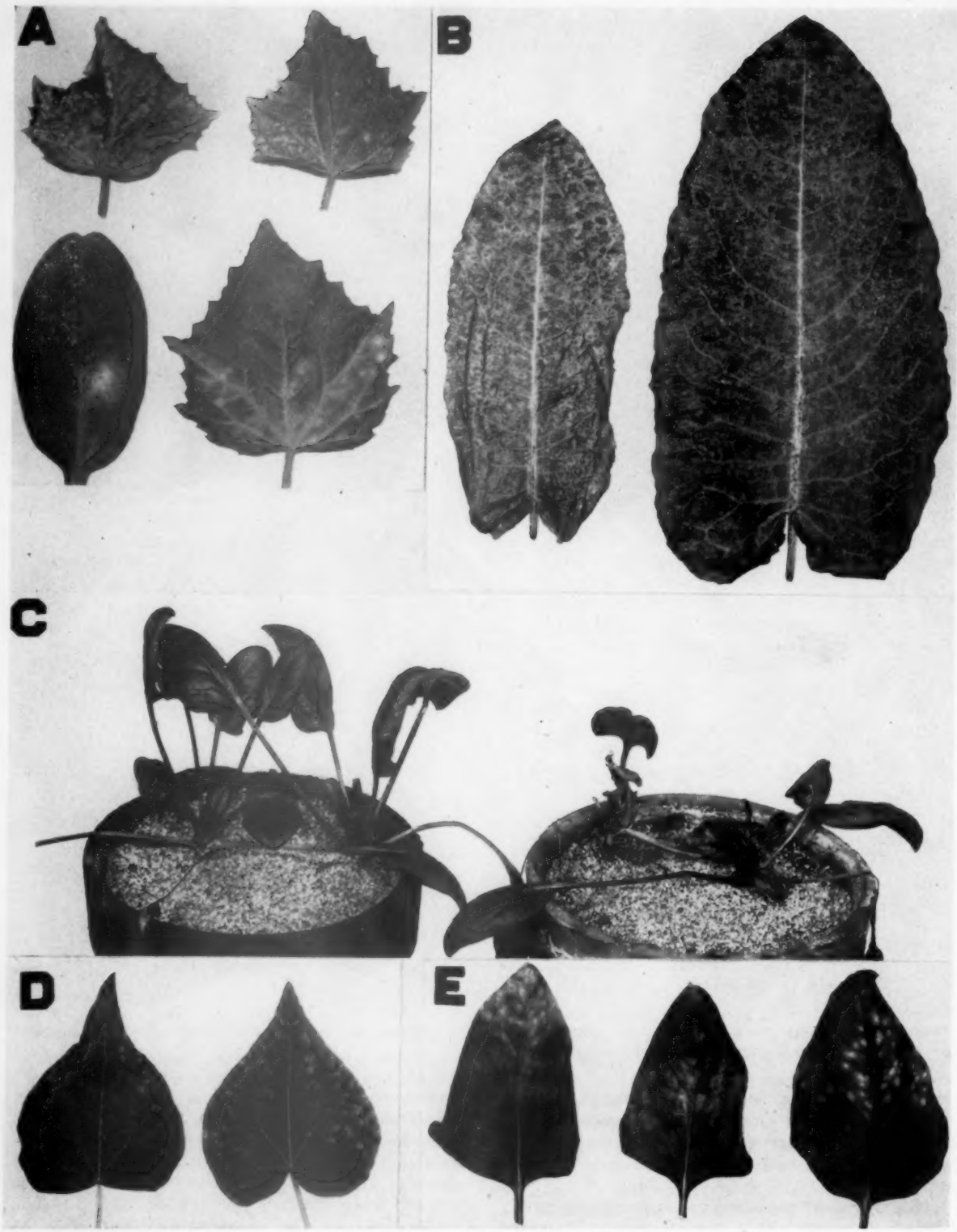

FIG. 2. Rhubarb virus in various hosts. A) Local lesion in cotyledon, chlorotic spotting, veinal and interveinal chlorosis in cucumber leaves. B) Mottle and chlorotic rings in curly dock. C) Right, yellowing, stunting, and deformation of spinach; left, controls. D) Yellow flecks in buckwheat. E) Chlorotic spotting and rings in spinach.

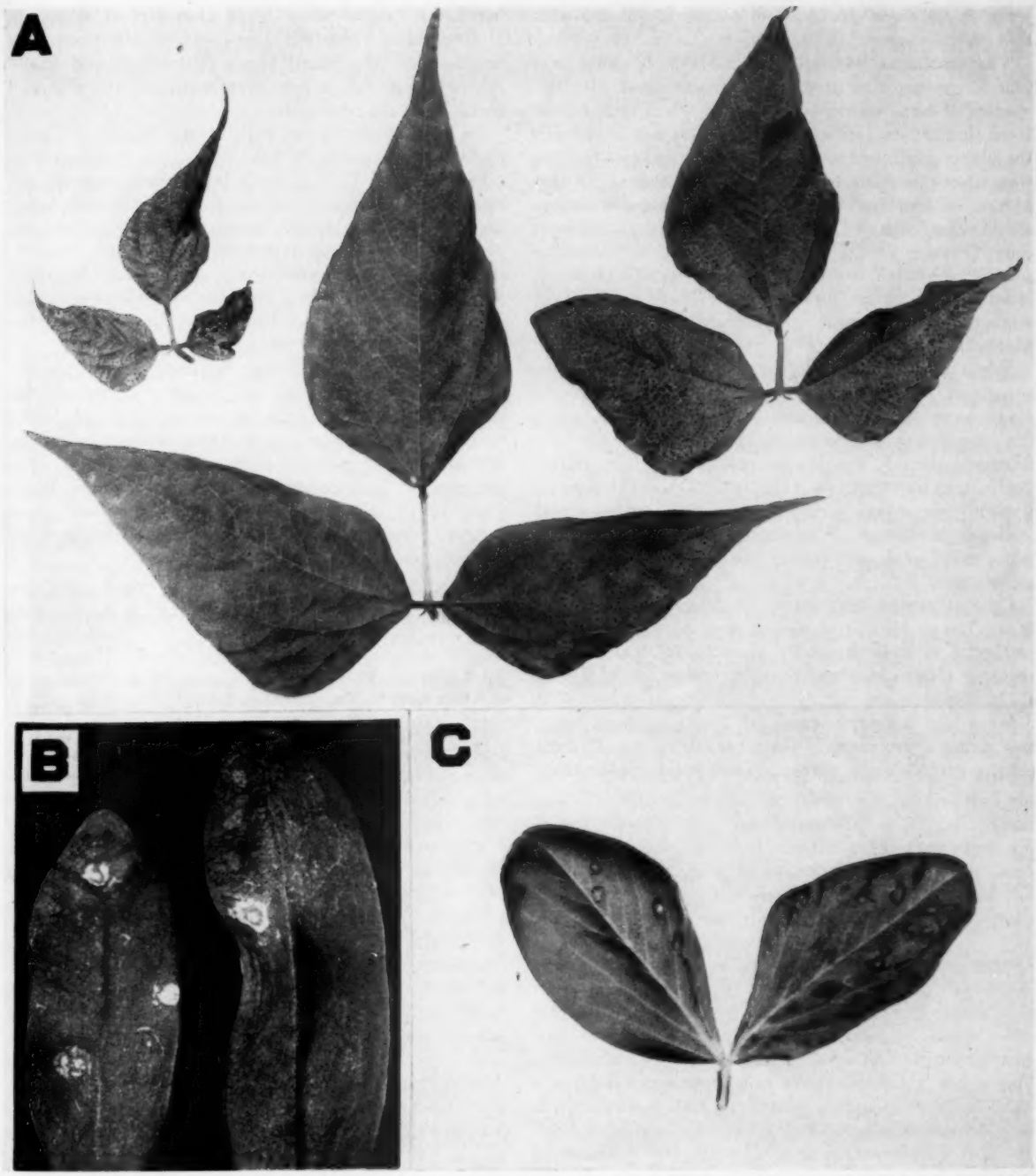

FIG. 3. Rhubarb virus in different hosts. A) Necrotic lesions with yellow halos and leaf deformation in Bountiful bean. B) Concentric necrotic ringspots in Sweet William. C) Necrotic lesions in broad bean.

spots, veinal and interveinal chlorosis, and became stunted and deformed (Figure 2, A). Growth ceased and the plants died a few weeks after inoculation. When the severe source was used, a higher percentage of transfers were successful and a greater number of chlorotic spots were produced than with the mild source, but the type of symptoms and their intensity were identical. Similar results were obtained when virus from the 2 sources was transmitted to spinach from rhubarb. Foliar symptoms in spinach were chlorotic spots and rings (Figure 2, E) and the leaves of some plants became yellow, stunted, curled, and finally necrotic. However, the plants continued to grow and subsequent leaves were either symptomless or had chlorotic spots and rings. Symptoms in spinach plants inoculated with the mild source were not dif-

ferent from those produced in plants inoculated with the severe source.

VARIATIONS IN BIOLOGICAL ACTIVITY.—In some test plants, the reaction to virus infection varied with the species of virus source plant used. When transmitted from rhubarb to spinach, the virus was not lethal and the plants continued to grow. However, when the virus was transmitted to spinach from buckwheat (*Fagopyrum esculentum* Moench), the spinach leaves became yellow, stunted, and curled and the plants soon died (Figure 2, C). Using cucumber as the virus source, concentric necrotic rings were formed in inoculated spinach leaves and subsequent leaves were yellow, stunted, and deformed. As before, all the infected plants died.

When buckwheat was infected by inoculation with sap from rhubarb, pale green mottle and chlorotic rings were observed in the leaves. However, when the virus was transmitted to buckwheat from artificially infected spinach, the leaves exhibited bright yellow flecks, but no ringspots (Figure 2, D). These comparisons were made at the same time under equal growing conditions. Apparently the source plant has some predisposing effect on the performance of the virus.

CLASSIFICATION.—Symptoms produced in Bountiful bean, broad bean and zinnia were similar to those produced in these hosts by known strains of tomato ringspot viruses obtained from cucumber by Dr. Frank P. McWhorter.

When the primary leaves of Bountiful bean were inoculated, the secondary leaves developed small, dark brown, necrotic spots surrounded by yellow halos (Figure 3, A). Subsequent leaves were stunted, distorted, and exhibited large necrotic spots in addition to the small ones. The plants began to defoliate and finally persisted with only a few severely stunted and yellowed leaves near the terminals.

In broad bean leaves, dark brown lesions, 1–2 mm. in diameter, some with light brown centers, were produced (Figure 3, C). Such leaves became generally necrotic as the lesions coalesced. Dark brown to black lesions appeared longitudinally on the stems and the plants usually wilted and died.

Chlorotic spots, interveinal and veinal chlorosis, slight necrotic stippling, and distortion were observed in zinnia leaves. Injury by the inoculum obscured the symptoms produced in inoculated leaves.

The symptoms on Sweet William (Figure 3, B) were sufficiently specific for ringspot[2] to place this virus provisionally in the genus *Annulus*. Infected tissues in living leaves of broad bean were examined by Dr. McWhorter using a high vacuum technic[3]. The presence of large alveolar granular viroplasts, characteristic of cells infected with ringspot viroses, gave further evidence that this virus should be classified in this group.

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[2] Weintraub, M. and J. D. Gilpatrick. 1952. An inhibitor in a new host of tobacco ring spot virus. Canadian Journal of Botany 30: 549-557.

[3] McWhorter, F. P. 1951. The examination of tissues in living leaves and flowers by means of a high vacuum technic. Stain Technology 26: 177-180.

TWO RECENTLY ISOLATED STRAINS OF CURLY TOP VIRUS [1]

N. J. Giddings [2]

SUMMARY

An extremely virulent strain of curly top virus has been discovered in sugar beets and in field collections of beet leafhoppers. It induces severe symptoms in some beet varieties that had been considered highly resistant and it is very destructive to tomatoes. It is most prevalent in southern Idaho but has been found in southern California. It has been designated as strain 11.

A curly top virus strain that readily infects commercial potato varieties was first obtained from eastern Washington in 1950 and from potato fields in southern California during 1952. It is also highly virulent in tomato. Tests from potatoes indicated that the virus concentration was rather low in them. This virus has been designated as strain 12.

Field collections of beet leafhoppers, *Circulifer tenellus* (Baker), are tested for presence of curly top virus by placing them individually on young sugar beet plants of a susceptible variety. This gives evidence as to the percentage of viruliferous insects and some indication of the virulence and classification of the virus strain or strains present (1). Additional evidence regarding the virus strains may be obtained by using both resistant and susceptible beets as test plants; the resistant variety S. L. 68 is often used. Both resistant and susceptible beet varieties were used in the work reported here.

STRAIN 11, HIGHLY VIRULENT TO SUGAR BEETS.—A new curly top virus strain was discovered in tests from beet leafhoppers collected in the Twin Falls, Idaho, area during the summer of 1947. Prior to this time curly top virus strain 1 had been the most virulent one tested on beet variety S.L. 68. The new strain or mixture of strains from the 1947 leafhopper collections induced symptoms in young plants of the resistant beet S.L. 68 that were nearly as severe as those in the susceptible variety S.L. 842 (Fig. 1). It was found that test plantings of that resistant variety in the field were also suffering very severely. Young plants of the usual susceptible varieties were quickly killed. While some of the newer beet varieties being developed for commercial use show a very much higher degree of curly top resistance than S.L. 68, this new virus strain or complex of strains injures them far more than any previously recognized strain.

Tomato plants of the variety Jubilee infected with this virus were killed in somewhat less time than plants of the same variety inoculated with other strains of curly top virus.

Inoculation tests with Burbank Russett potato gave only 8 plants infected out of 49 inoculated, 16 per cent, indicating low virulence toward potato.

The virus strain or complex associated with the production of these severe symptoms has been repeatedly encountered in leafhopper collections and in sugar beet plants from Idaho. Similar virus has also been found in the southern California beet growing areas. This virus has been maintained in both susceptible and resistant hosts for more than 4 years without evident change in virulence and is designated as curly top virus strain 11.

The discovery of this curly top virus strain is a clear-cut warning that there may be other highly virulent strains developing and appearing in sugar beets or other crops. Much remains to be accomplished in developing resistant sugar beets.

STRAIN 12, VIRULENT TO POTATO AND TOMATO.—Potatoes and tomatoes thought to be curly-top infected were sent to this laboratory from Prosser, Washington, during 1950-51 by Dr. J. D. Menzies of the Division of Soil Management and Irrigation of the United States Dept. of Agriculture. When tested curly-top virus was obtained from nearly every specimen. Curly top on potatoes was reported by Severin in 1929 (7), and by Jones, Vincent, and Burk in 1940 (4) but the writer had tested more than 200 suspected potato plants from commercial fields during a 10-year period and had obtained no curly-top virus from any of them. These tests included plants of several potato varieties obtained from Oregon, Idaho, southern California, and Arizona. It was therefore surprising, in spite of the earlier reports, to obtain curly-top virus from the specimens sent by Dr. Menzies.

Test plants that were considered very susceptible to curly top were usually quite slow in developing symptoms when inoculated by means of leafhoppers that had fed on diseased potato plants. It would seem either that the virus concentration in the potato was low or that the sugar beet variety used was not a very favorable host for this strain. Four colonies of leafhoppers carrying virus from these potato sources were established on susceptible beet plants during 1951; in less than a year 3 of the colonies had become nonviruliferous. Some colonies carrying highly virulent virus had become nonviruliferous in earlier experiments (2) but there have been no cases comparable to this one. This would suggest that the beet is not a favorable host but test plants inoculated from beets usually develop symptoms sooner than similar plants inoculated from potato, which might indicate a higher virus concentration in the beet than in the potato.

Symptoms induced in test beet plants are somewhat similar to those induced by virus strains 5, 6, and 10 (1). There is very little veinclearing and sometimes

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[2] Senior Pathologist, U. S. Dept. of Agr., Sugar Plant Investigations, Riverside, California.

FIG. 1 A) Top row is sugar beet variety S.L. 68 and bottom row is S.L. 842. Plants in pots at left infected with curly top virus strain 1, in two middle pots with strain 11, and in bottom pot at right with strain 12. Plants in pot at top right are healthy controls. B) Close up of sugar beet plant infected with strain 12, showing lateral rolling of leaves and prominent veins.

practically none of the leaf symptoms originally associated with curly top on sugar beet. The leaves have a tendency to roll laterally, but healthy plants of some beet varieties may show a similar tendency. The color of infected plants is likely to be darker green in the early stages. Veins on the underside of the leaf usually show some swelling or enations and the plants are commonly much dwarfed (Fig. 1).

Infected tomato plants of the variety Jubilee were injured as severely, and killed as quickly as those infected with virus strain 11.

The most important host relationship thus far discovered is that young potato plants grown from certified Russett Burbank tubers showed more than 50 per cent infection after inoculations using 5 or more viruliferous leafhoppers per plant. It is comparatively difficult to obtain as much as 10–20 per cent infection using similar plants with other strains of the curly-top

virus and as many as 50 leafhoppers per plant. The finding of this virus strain that is especially virulent toward potato helps to clarify some of the difficulty and confusion regarding curly top in potatoes.

This virus strain occurs in Washington, Oregon, and southern California. In Oregon, Milbrath reported it as green dwarf (6) in 1946; its identity with curly top was reported by Menzies and Giddings in 1953 (5). It was first identified in southern California in 1952 by Giddings (3), although there is good evidence that it was present in that area at least as early as 1950.

This virus has been designated as curly-top virus strain 12.

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LITERATURE CITED

1. GIDDINGS, N. J. 1944. Additional strains of the curly top virus. Jour. Agr. Res. [U.S.] **69**: 140-157.
2. GIDDINGS, N. J. 1950. Some interrelationships of virus strains in sugar beet curly top. Phytopathology **40**: 377-388.
3. GIDDINGS, N. J. 1954. Some studies of curly top on potatoes. Phytopathology **44**: 125–128.
4. JONES, L. K., C. L. VINCENT, AND E. F. BURK. 1940. The resistance of progeny of the Katahdin potatoes to viroses. Jour. Agr. Res. [U.S.] **60**: 631-644.
5. MENZIES, J. D. AND N. J. GIDDINGS. 1953. Identity of potato curly top and green dwarf. Phytopathology **43**: 684–686.
6. MILBRATH, J. A. 1946. Green dwarf, a virus disease of potatoes. Phytopathology **36**: 671-674.
7. SEVERIN, H. H. P. 1929. Additional host plants of curly top. Hilgardia. **3**: 595-637.

SOME STUDIES OF CURLY TOP ON POTATOES [1]

N. J. Giddings [2]

SUMMARY

Potato seedlings of the variety, Earlaine, self fertilized, are highly susceptible to infection, under greenhouse conditions, by any of the more virulent strains of curly top virus. Many infected plants failed to produce tubers. In some cases no virus was obtained from any of the tubers produced by an infected plant but in most cases the virus was recovered from all, or nearly all, of the tubers.

Tests of many suspected plants from commercial fields gave entirely negative results until 1950. Inoculations of and tests from many plants of commercial potato varieties gave erratic results and comparatively rare infections until 1951. The curly top virus strain 12, isolated in 1950 infected commercial potato plants readily and has caused appreciable economic damage in fields being grown for certified seed stock.

Movement of the virus through potato stems appeared to be slow; the virus did move from an infected stem into the tuber and into other stems that originated as much as 2 in. from the inoculated stem.

The potato may help to identify strain 12 but otherwise it is not a good host for differentiation of curly top virus strains.

The work reported in this paper was undertaken first with the hope that potato seedlings might be good differential hosts for some of the curly top virus strains then known. Commercial potato varieties were then used in similar tests and, finally, experiments were conducted to try and learn why there was so much irregularity and uncertainty in obtaining curly top infection of such plants.

In 1929 Severin (7) reported obtaining curly top from potato plants in 1925. He also reported infecting plants of several potato varieties but did not determine whether there was tuber transmission of the virus.

Jones, Vincent, and Burk (4) reported 8–10 per cent curly top in Katahdin seedlings near Pullman, Washington. Tubers from 25 of the plants believed to be curly-top infected were planted. Plants from only 3 tubers developed symptoms suggestive of curly top, and tomato cions grafted onto these developed typical curly top symptoms. They stated that evidences of appreciable curly top injury coincided with the use of Katahdin as a parent in the seedlings. Milbrath described Green Dwarf of potato in 1946 (6). He reported that it was tuber transmitted and that it was a problem in the production of certified seed stock in Central and Eastern Oregon. He also commented on the prolonged dormancy of infected tubers and the abnormal, stunted plants that develop from them.

In 1947 Giddings (2) reported some curly top studies on potato and that the commercial potato varieties tested were quite resistant to infection. Menzies and Giddings (5) have shown that the curly top virus induces Green Dwarf symptoms in potato, and reported greatly prolonged dormancy of infected tubers.

Separation and classification of sugar-beet curly-top virus strains require the use of differential hosts. The sugar beet variety S. L. 68 and Turkish tobacco have been very useful in this connection, so work was undertaken with seedling potatoes as possible differential hosts. After Jones et al (4) reported that seedlings from the Katahdin variety appeared to be particularly susceptible, additional work was undertaken using plants grown from tubers as well as seedlings.

In earlier experiments all available curly top virus strains were used for inoculations. There were no significant differences in the amount of infection obtained except that the less virulent strains 2, 4, and 7, very rarely induced infection. For that reason these 3

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TABLE 1.—*Tubers produced by curly-top-infected potato plants and results of tests from plants produced by such tubers*

	Number of tubers produced per plant						
	1	2	3	5	7	12	15
Number of plants:							
Harvested	11	2	4	1	1	1	1
Grown from uncut tubers	7	3	12	3	6	8	12
Virus recovered from	6	3	10[a]	3	5	0	0

[a] The plant produced by 1 tuber from each of 2 parents failed to yield curly top virus when tested on sugar beets.

strains were seldom used and all data dealing with commercial potato varieties are based on experiments including only the more virulent strains 1, 3, 5, 6, 8, and 9.

About 400 potato plants and tubers from Arizona, California, Idaho, and Oregon have been tested for curly top virus in an effort to learn whether the disease was present, and, if so, whether the virus was different from other known strains affecting sugar beets. Approximately 200 of these tests were made previous to 1950; curly top virus was not obtained from any of the specimens, although many were carefully selected in the field from plants that showed symptoms suggestive of curly top.

EXPERIMENTS WITH POTATO SEEDLINGS.—All seedling plants were grown from seed of the variety Earlaine, self fertilized. Forty-four plants were inoculated in 1939. Curly top virus was recovered and transmitted to sugar beet from 3 of the plants showing curly-top-like symptoms and from 3 of the others.

During tests made in 1943, 117 seedlings were inoculated; 35 of these showed symptoms recorded as curly top, 20 as doubtful, and 62 as negative. Curly top virus was recovered from 31 (89 per cent) of the first group, 8 (40 per cent) of the second group, and 3 (5 per cent) of the third group. This evidence suggests that there is a reasonable probability of recognizing curly top infection under greenhouse conditions.

The plants in 1 group of 44 infected seedlings were harvested about 4 months after inoculation. Twenty-three of the plants produced no tubers. Tubers produced by the remaining plants were stored for about 4 months and the uncut tubers planted in the greenhouse. Plants from the latter were tested for curly top virus by leafhopper transfer to sugar beet (Table 1).

Results from this experiment suggest that there is a strong tendency for the virus to be transmitted in all or none of the tubers from an infected plant. The failure of more than half the plants to produce any tubers also suggests that infection of the young plant may interfere with tuber production. The seedling plant that produced 15 tubers was infected by the least virulent virus strain available and virus was recovered from the original seedling but from none of the progeny.

Of 74 seedling plants inoculated and tested during 1946-47, curly top virus was recovered from 24.

EXPERIMENTS WITH COMMERCIAL POTATO VARIETIES.—Jones et al (4) had reported Katahdin seedlings susceptible, so plants were grown from certified seed stock of that variety. Eleven were started in a small outdoor plot and 30 in an unheated screened house in April 1943. Fifteen plants of Russet Burbank were also used in the screened house. One sprout 4–8 in. long was inoculated on each plant, using 15 viruliferous leafhoppers per plant and leaving them on for 16 days. Inoculations were made in May and the plants tested 1 month later. Whenever possible, tests were made from the inoculated sprout and in several cases additional tests were made from another nearby sprout. Curly top virus was not recovered from any of the 56 plants.

In the unprotected outdoor plot, 5 plants were selected, each having a strong sprout with a good side branch ½–⅔ of the distance up from the soil. The main sprout of each plant was inoculated using 40 viruliferous leafhoppers. Six days later 20 more viruliferous leafhoppers were added to each of these cages and nonviruliferous leafhoppers were caged on the side branch just below the portion covered by the inoculation cage. All leafhoppers were removed 5 days later and the nonviruliferous leafhoppers were tested on young susceptible sugar beet plants. None of the test plants became infected.

During 1944, greenhouse inoculations included 22 Katahdin and 20 Russet Burbank plants. Thirty to 40 viruliferous leafhoppers were caged on each young plant. All plants were tested 1 or more times and curly top virus was recovered from 2 plants of each variety.

Similar inoculations were made during 1945 on 17 Katahdin and 17 Russet Burbank plants, using at least 50 leafhoppers per plant. Virus was recovered from 2 plants of each variety.

Inoculations were made in field plots of Katahdin and Russet Burbank in the spring of 1945, of White Rose in the fall of 1945, and of White Rose in the spring of 1946. There were 2 rows approximately 110 ft. long and 3 ft. apart; the plants were 1 ft. apart in the row. Each row was divided into 8–10 plots and an attempt was made to have approximately the same number of plants in each 2 consecutive plots in a row. Each plot was covered with a muslin tent about 18 in. high and 2 ft. across at the point of contact with soil. The edges of the cloth were covered with soil.

Alternate plots in each row were inoculated when the plants were 4–10 in. tall, using approximately 50 viruliferous leafhoppers for each plant. The leafhoppers were a mixed group from colonies carrying all the more virulent curly top strains available at that time. Inoculated and noninoculated plots were thoroughly dusted with 10 per cent Nicodust 2 weeks after placing the leafhoppers in the tent cages. When the

cloth tents were removed the next day, no living leafhoppers were found.

From the 1945 field, 5 suspected plants of Russet Burbank and 25 of Katahdin were carefully tested. No curly top virus was recovered from any of the plants. In the 1946 field plots 53 plants were tested and the presence of virus was established in only 3 of these by successful transfer to sugar beets. These results clearly indicated that the potato varieties used were difficult to infect with the curly top virus strains then available.

Yields from inoculated plots.—Yield data were obtained from each of the field plots inoculated during 1945 and 1946. It was thought that there might be some effect upon tuber development even when symptoms were not evident above ground.

In the entire series of 3 plantings there were 4 inoculated plots of Russet Burbank, 4 inoculated plots of Katahdin, and 16 inoculated plots of White Rose potatoes. For each inoculated plot there was an adjacent and comparable noninoculated plot. In no case was there any significant difference in the yield from inoculated plots and noninoculated plots.

In view of Menzies' data (5) from Prosser, Washington, the failure to influence yield may have been due to the virus strains involved and the small amount of infection.

Virus movement through the tuber.—Many of the inoculation experiments, including the 1946 field inoculations, were made upon plants developed from 1 eye of a tuber. Sometimes the inoculated shoot would be injured or die, and the single eye gave assurance that any other sprout tested originated adjacent to the inoculated one.

Pieces of tuber containing 2 or 3 eyes also were planted to determine possible movement of virus through the tuber, from 1 eye to another. Infection of the inoculated shoot was obtained in 12 cases, as proved by transmission to sugar beet. Four infected plants had noninoculated portions of the plant covered with a dark chamber for a week and were tested at intervals beginning a week after the covers were removed and ending 6 weeks later. The covered portions included side branches from the inoculated stem, independent stems originating from the same eye, and branches originating from other eyes in the tuber piece. Virus was not recovered from any of the covered branches 3 weeks after covering, but was recovered from the side branches of 3 plants 5–6 weeks after covering. At 6 weeks virus was also recovered from an independent stem originating in the same eye as the inoculated stem and from 1 branch originating in an eye 1½ in. from the noninoculated stem.

Another group of 8 infected plants was allowed to grow in normal greenhouse light, and occasional tests were made from side branches of the infected stalk and from independent stems. Virus was not recovered from side branches until about 6 weeks after inoculation; 9 weeks after inoculation virus was recovered from a stem that originated in an eye 2 in. from the inoculated one.

These experiments indicate that the curly top virus moves rather slowly in potato plants, and that it may move down through an infected stem and the seed tuber to other stems originating some distance from the originally infected one. Limited evidence from 1 experiment indicates that enclosing a noninfected branch or stem in a dark carton for a week does not appreciably accelerate the movement of the virus into the covered portion.

TRANSMISSION BY GRAFTING.—Reciprocal grafts were made between potato and *Nicotiana tabacum*, *N. glutinosa*, *Datura stramonium*, and *Lycopersicon esculentum*. Some inoculations were made through the cion, and some through the stock, using viruliferous leafhoppers. Others were made by using cions from infected plants of Turkish tobacco, tomato, or potato that had partially recovered after showing pronounced symptoms. The infected Turkish tobacco cions seemed to be the most satisfactory although good results were obtained from potato on tomato and tomato on potato. Twenty of the graft inoculations using tobacco cions gave 16 infected plants. Inoculations of the healthy cions of Pearson tomato on Russet Burbank potato gave only 2 infections out of 8. The numbers of plants involved are too small to be significant, but the results are suggestive.

RECENT EVIDENCE OF CURLY TOP INFECTION IN COMMERCIAL FIELDS.—Late in 1950 Dr. J. D. Menzies of the Irrigation Experiment Station, Prosser, Washington, sent to this laboratory specimens of potatoes suspected of being infected with curly top virus. Curly top virus was obtained from these specimens and from others received from Prosser in 1951, as reported by Menzies and Giddings (5).

A large field of potatoes grown for seed stock near Rosamond, Calif., during 1952, was first seen by the writer in August; at that time they showed symptoms similar to those of curly top as described by Severin (7) and Milbrath (6). Numerous plants carried axillary branches that were dwarfed, with small leaves clinging close to the stem. There was also evidence of yellowing foliage with purplish discoloration at the tips of the stalks. The writer collected specimens from plants that appeared to have symptoms most suggestive of curly top infection. A second collection was made about 1 month later by Dr. R. A. Flock of the Citrus Experiment Station, University of California. Curly top virus was transmitted to sugar beet from 14 plants out of 25. It was estimated that 8–10 per cent of the plants in the field were curly top infected.

Another large field of potatoes near Tehachapi, Calif., about 25 mi. north of the one previously mentioned was examined Oct. 22, 1952, when the crop was nearly ready for harvest. The plants were extensively yellowed and many showed symptoms similar to those in the Rosamond field. Specimens were collected and tested for curly top virus, which was found in 9 plants out of 25.

Of the 50 plants collected from the 2 fields because they showed symptoms typical of curly top, only 23 plants produced recoverable virus. This indicates that curly top symptoms are not readily distinguished in the field, where many other factors influence growth of the plant, or where there may be complications due to the presence of other virus diseases. It is also possible that some symptoms were induced by curly top virus even when no positive evidence was obtained with tests on sugar beet plants.

Discussion.—The 2 fields where curly top was found in the fall of 1952 were very close to range and desert areas in which there may well be extensive winter breeding grounds for the beet leafhopper, *Circulifer tenellus* (Baker). One grazing area about 15 mi. southwest of the Rosamond field was examined in mid-December 1952; beet leafhoppers were abundant on winter annuals. In some seasons there might be a heavy movement of leafhoppers from such breeding areas into the potato fields while the plants are comparatively young. When the potato specimens were collected at Rosamond and Tehachapi, Dr. R. A. Flock collected beet leafhoppers in each of the fields although these insects were comparatively rare then.

When inoculated into susceptible sugar beet plants, the curly-top virus strain, or strains, obtained from potatoes in the Rosamond-Tehachapi area induced symptoms similar to strains 5, 6, and 10 (1) and behaved much like strain 12 (3) obtained from potatoes in eastern Washington. In 1938 curly top virus was obtained from a suspected specimen of the California poppy, *Eschscholtzia californica* Cham., collected near the Rosamond-Tehachapi area. In susceptible sugar beets this virus induced symptoms similar to those induced by the virus recently obtained from potatoes. This strain of the virus may be increasing on poppies and other wild hosts and may be the one causing so much damage to potatoes now.

Thornton (8) reported seeing typical cases of curly top, then known as green dwarf, in the Cuyama Valley west of Maricopa, California, during 1950, and in the Arvin-Edison area, about 20 mi. northwest of Tehachapi, during 1951. Tubers from each field were grown in the greenhouse and plants showing the typical curly top (green dwarf) symptoms were obtained from each group. He also made a large number of grafts from infected plants to healthy plants and obtained transmission in a few cases. It is well known that some seed stock used in these areas is obtained from the Northwestern United States and Thornton's report would indicate that the disease may have been introduced in that way.

It is probable that curly top infection of growing potato plants would adversely affect both the yield and quality of tubers produced. Infection with curly top virus has been reported as adversely affecting yield of potatoes in Prosser, Wash. (5), but adequate confirming data are not available from California areas where curly top has been found recently. Virus strain, potato variety, age of plants when infected, and environmental conditions may well be important factors in determining the effect on yield. It is known (5, 6) that many tubers from infected plants are extremely slow in sprouting and produce abnormal plants with very poor yields.

There are very few data concerning the resistance of potato varieties, the virus strains involved, or the actual economic importance of the disease on potatoes. The curly top infection caused an estimated $15,000 reduction in the value of the crop from the 2 fields investigated in 1952, as they had been grown for certified seed stock and so much curly top disqualified them. Since the virus is carried in the tubers it is important to know whether early or late infection of the plant makes any difference as to the number of tubers infected and the type of injury that may become apparent as a result of such infection. The virus seems to move rather slowly in the potato and the concentration appears to be low because of the relatively long time usually required for beet plants to develop symptoms when inoculated from diseased potatoes.

Infected potato plants in the greenhouse may show symptoms and normal appearing leaves alternately on the same stem as growth progresses, a possible factor in complicating symptoms in the field. Jones, Vincent, and Burk (4) stated that symptoms of aster yellows and of Psyllid yellows might be confused with curly top. There is urgent need for the discovery or recognition of symptoms or tests that will enable certain recognition of this disease.

These studies of curly top virus did not indicate that potatoes would be helpful as a differential host for the virus strains previously known but did bring to light another new and very important strain of the virus (3).

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LITERATURE CITED

1. Giddings, N. J. 1944. Additional strains of the curly top virus. Jour. Agr. Res. [U.S.] **69**: 149-157.
2. Giddings, N. J. 1947. Some studies on curly top of potato. (Abs.) Phytopathology **37**: 361.
3. Giddings, N. J. 1954. Two recently isolated strains of curly top virus. Phytopathology **44**: 123–125.
4. Jones, L. K., C. L. Vincent, and E. F. Burk. 1940. The resistance of progeny of Katahdin potatoes to viroses. Jour. Agr. Res. [U.S.] **60**: 631-644.
5. Menzies, J. D. and N. J. Giddings. 1953. Identity of potato curly top and green dwarf. Phytopathology **43**: 684–686.
6. Milbrath, J. A. 1946. Green dwarf, a virus disease of potatoes. Phytopathology **36**: 671-674.
7. Severin, H. H. P. 1929. Additional host plants of curly top. Hilgardia. **3**: 595-629.
8. Thornton, R. L. 1953. From personal correspondence, dated May 12, 1953.

DIEBACK AND CANKER OF CAMELLIAS CAUSED BY GLOMERELLA CINGULATA [1]

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SUMMARY

A disease of camellias, characterized by the formation of cankers and the dying back of twigs and branches, is described.

The results of this study confirm the findings of previous investigators that the incitant of the disease is the fungus *Glomerella cingulata* (Stone.) Spauld. and von Schrenk.

The fungus is incapable of causing infection in nonwounded tissues. In nature, infection occurs principally through leaf scars in early spring. Infection may also occur through bark wounds made by cultivating implements, lawn mowers, and freeze injuries. In grafted plants, infection often starts at the graft union.

Infection, resulting in typical symptoms, was produced artificially with pure cultures of the fungus by applying the inoculum to incisions in the bark, to needle punctures in shoots, and to scars of 1) naturally abscised leaves, 2) leaves induced to abscise by deblading, and 3) leaves removed mechanically.

The varieties tested varied in degree in their susceptibility to the disease. Some varieties (Governor Mouton, Professor Sargent) showed considerable resistance, while others (Flora Plena, Prince Eugene Napoleon) were highly susceptible.

Differences in pathogenicity among isolates of the fungus were noted. Several isolates were nonpathogenic.

Dieback is one of the most common diseases affecting camellias in the Southern States. Observations made at nurseries and private gardens have revealed the widespread occurrence of the disease in Louisiana. In other Southern States investigators (1, 6, 10) have reported the disease as being general and of considerable economic importance. It has also been reported by Davis and Pirone (2) as occurring on greenhouse-grown camellias in Ohio, Pennsylvania, and New Jersey.

There has been considerable disagreement in regard to the cause of dieback. Post (8) was apparently the first to report that a fungus belonging to the genus *Gloeosporium* was the cause of the disease. She successfully reproduced the disease by inoculating camellia plants in wounds with pure cultures. Bain (1), also, isolated a Gloeosporium from camellias affected with dieback and cankers, and succeeded in producing infection by introducing a conidial suspension of this fungus into punctures in young shoots. Davis and Pirone (3) isolated *Glomerella cingulata* (Stoneman) Spaulding and von Schrenk from camellia cankers, and induced wilting of young shoots by inoculating in wounds with monoascospore isolates of this fungus. West (9) found *Phomopsis*, *Diplodia*, and *Gloeosporium* consistently associated with dieback in Florida, but was unsuccessful in inducing infection with any of these fungi. Plakidas (7) (and Plakidas, unpublished data) isolated a Colletotrichum consistently from dieback and canker material. He was unable to reproduce the disease in healthy plants growing under normal conditions by inserting the inoculum in incisions under the bark, but did obtain cankers in plants first rendered weak and unthrifty by such means as root pruning or flooding.

The present study was undertaken to obtain more definite information regarding the cause of, and the factors favoring, initiation and development of dieback and canker of camellias.

DESCRIPTION.—As the symptoms vary considerably with age and size of the organ affected, the different phases of dieback and canker are described separately.

Dieback of young growth.—This phase occurs in early spring during the first flush of new growth. Young, succulent shoots, terminal or lateral, wilt and die rather suddenly. The dead leaves become dark brown to black and usually remain attached to the wilted shoot for some time. At the junction of the necrotic and the living portions of the shoot there is an area an inch or more in length in which the bark as well as the woody tissue is dark brown. A *Colletotrichum* can invariably be isolated from these discolored tissues. Infection responsible for this type of injury often occurs at the base of the new shoot, i.e., at the junction of the new growth with the old, but occasionally may occur at some distance above the base. A similar type of dieback occurs in grafted plants, the infection apparently originating at the graft union.

Dieback of twigs and small branches.—This phase is similar to that described above in that there is sudden wilting and dying back of twigs and small branches, but the wilting occurs at any time during the season and is not restricted to the early spring. Cankers almost invariably accompany this type of dieback. The cankers are either inconspicuous, slightly depressed areas in the bark at the base of the necrotic portion of the twig or branch or sunken and well defined (Fig. 1, A, B, & C). The cankers range from about 0.5 to 3.0 cm. in diameter, depending on the size of the affected twig or branch. Usually the cankers are localized either at the base of the dead twigs where infection occurred through the leaf scars, or on the branches, developing around the necrotic remnants of twigs which had been killed earlier.

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Dieback of larger branches and of whole plants.—This is the most destructive type of dieback. It entails the loss of large branches or of the entire plant when the main stem is girdled. Definite cankers are present. Death occurs only when the branch or stem has been girdled, whereas partially girdled branches often show signs of distress—chlorotic, sparse foliage and dieback of small twigs. On main stems, cankers usually develop around bark wounds made by cultivating implements or lawn mowers, and in the case of grafted plants at the graft union. Also, there is some indication that injuries to the bark caused by freezing may afford entry for the fungus. This usually occurs on the main stem, particularly near the ground level.

Other diseases, as well as physical factors such as drought or flooding, may produce symptoms somewhat similar to those of dieback. A root rot described by Gill (5), caused by *Phytophthora cinnamomi* Rands, produces symptoms which may be mistaken for those of dieback. The foliage becomes sickly, chlorotic, and sparse, and there is a gradual dying back of twigs and branches. However, differing from dieback, the organism involved in root rot is confined to the root system and cankers are not produced on the branches and stems. Diagnosis is further complicated by the fact that a plant may be affected by both dieback and root rot. Plants weakened by root rot or by some other adverse factor such as drouth, waterlogging of the soil, severe pruning of the roots at the time of transplanting, or freeze injury, appear to be more susceptible to dieback.

EXPERIMENTAL RESULTS.—*Isolations.*—To determine what organisms are associated with dieback, dead or dying twigs and branches, most of which bore cankers of various sizes, were collected throughout the year from several locations in the state. Isolations were made from more than 50 varieties of *C. japonica* and also from 2 other species of camellia, *C. sasanqua* and *C. reticulata.* Small bits of the discolored bark and wood were plated on potato-dextrose agar. Approximately 5000 isolations were made, and about 80 per cent yielded pure cultures of *Colletotrichum.* Other fungi isolated more or less frequently included *Pestalotia* spp., *Fusarium* spp., and *Diplodia theobromae.* The *Colletotrichum* was isolated from diseased material from all 50 varieties of *C. japonica* and from the *C. reticulata* and *C. sasanqua* specimens, and was therefore the one organism found constantly associated with the disease.

In preliminary studies, inoculations were made on 3 camellia varieties, using pure cultures of the *Colletotrichum, Fusarium, Pestalotia,* and *Diplodia.* The inoculum, consisting of bits of mycelium, was inserted under the bark of mature branches. Infection was obtained with the *Colletotrichum* but not with the other fungi.

After it was demonstrated that the *Colletotrichum* was capable of infecting camellias and causing dieback and cankers, additional inoculations were made using the following techniques of applying the inoculum. (Table 1.)

TABLE 1.—*Summary of the results obtained by different methods of inoculation*

Method of inoculation[a]	No. varieties tested	Isolates tested[b]	Results[c]
A. Nonwounded			
1	3	4/4	0/50
2	3	2/2	0/45
3	2	2/2	0/45
4	3	2/2	0/40
5	9	2/2	3/44
B. Wounded			
1	22	9/12	803/1136
2	6	1/1	14/35
3	7	2/2	42/47
4	18	4/5	207/319

[a] Method of inoculation: A1—Conidia applied to swelling buds; A2—Conidia applied in axis of stem and branch (old growth); A3—Conidia applied in axis of stem and branch (new growth); 4A—Conidia applied in axis of leaf and stem (new growth); A5—Conidia atomized on nonwounded plants; B1—Conidia applied to scars of mechanically removed leaves; B2—Conidia applied to scars of leaves induced to abscise by deblading; B3—Conidia applied to scars of naturally abscised leaves; B4—Conidia applied through mechanical lesions.

[b] The denominator of the fraction gives the number of isolates tested, the numerator the pathogenic isolates.

[c] The denominator of the fraction gives the number of branches inoculated, the numerator the number infected.

Inoculation without wounding.—Several experiments were carried out to determine whether or not the fungus was capable of causing infection in nonwounded tissue. In 1 test, 44 1-year-old plants, consisting of 9 varieties growing in 2-gallon crocks, were placed in a large moist chamber. A quantity comprising 4 l. of a conidial suspension of the fungus was connected to a compressed air line and atomized slowly on these plants for a period of approximately 20 hours. The plants were left in the moist chamber for an additional 48-hour period and then removed. The test was made when the new growth was young and tender. Only 3 of the 44 plants developed dieback during the subsequent 3-month period, and in each case the infection was traced to a wound which had escaped detection when the plants were selected.

In further studies, a conidial suspension was applied to the swelling vegetative buds at the time of new growth in the spring. Bain (1) had expressed the idea that "infection takes place at or about the bud scale scars shortly after the buds begin to swell early in the season." Fifty buds of 3 varieties were thus inoculated. Moist cotton was wrapped around the inoculated buds and kept moist for at least 12 hours. None of the plants became infected.

In other tests, inoculum (conidia) was applied to the crotch between the lateral branches and to the main stem (both old and young growth) and at the junction of leaves and shoots (both old and young shoots). In every case moist cotton was wrapped

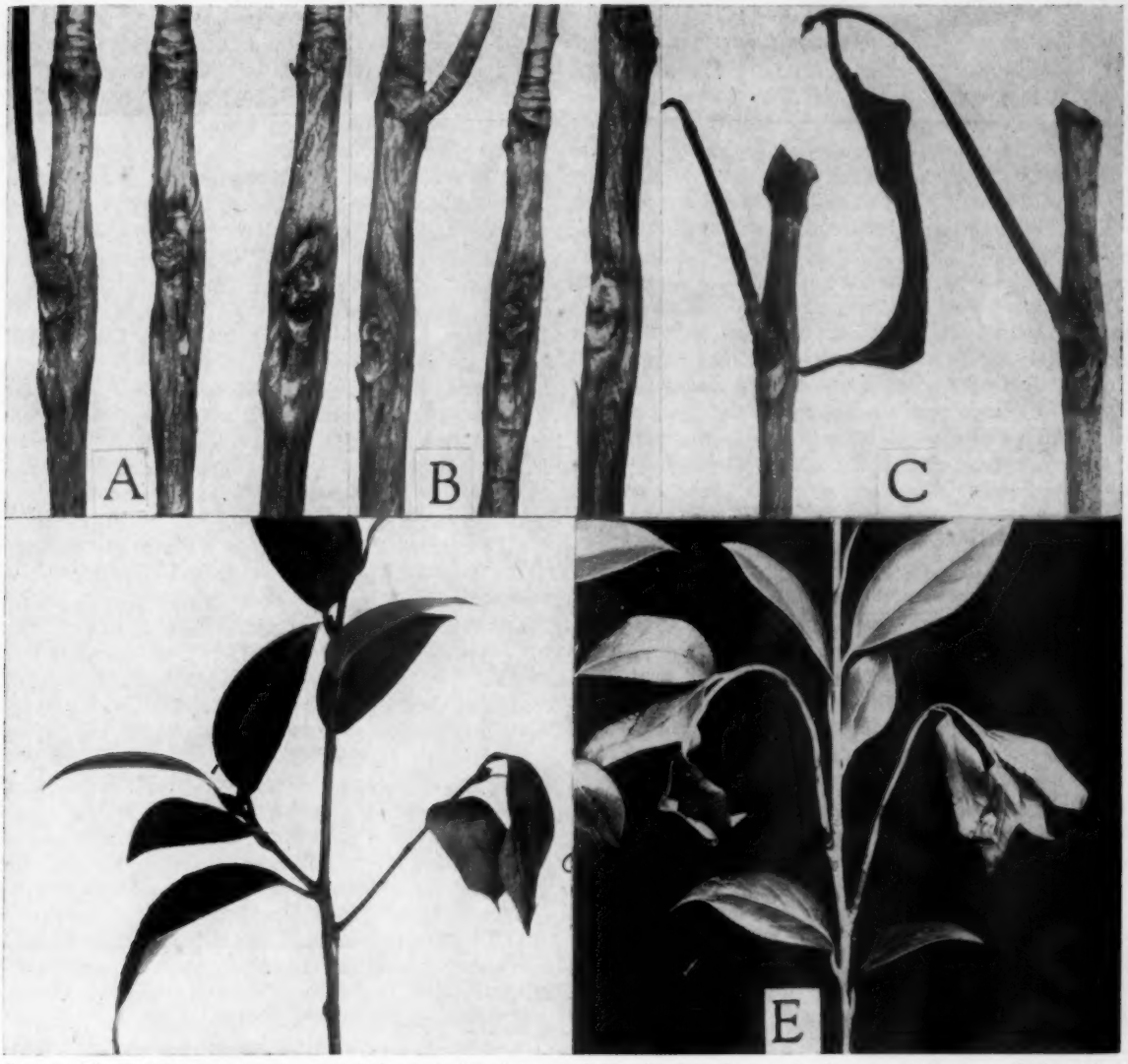

FIG. 1. (A - B) Cankers on 2-year old shoots induced by applying the inoculum to leaf scars. A) variety Rubra Virginalis; B) variety Altheaflora. C) Natural infection through leaf scars on 1-year old shoots. D, lower left) Natural infection on current-season's twig. E) Infection on 2 young shoots induced by inoculation through leaf scars.

around the inoculated area and kept wet for several hours. In no case was infection obtained. These results indicate that the fungus is incapable of invading nonwounded tissue, regardless of whether the tissue is that of mature wood or of succulent new growth.

The needle prick method.—Young succulent shoots, and also shoots of mature wood, were punctured with a sharp needle and a conidial suspension was applied with a brush to the punctured areas. Plants of 2 varieties, Sarah Frost and Prince Eugene Napoleon, were used. A total of 219 shoots of the former variety and 100 of the latter variety were inoculated. Infection resulted in 207 of the 319 inoculated shoots. Both young and old growth became infected. Cankers developed first at the points of inoculation and then the distal portions of the shoots began to die back. Dieback developed after about 1 month on the young growth and after several months on the old shoots.

Inoculations through leaf scars.—It has been observed that most natural infections occur on or around the scars left by abscising leaves. Subsequent to infection through the leaf scar, young lateral twigs are killed followed by the formation of a canker encircling the twig. The terminal portion of the branch may also be killed if it is girdled by the canker. To confirm these observations, a series of experiments were conducted in which the inoculum, in the form of conidial suspension, was applied to leaf scars. Three different techniques were used in obtaining and selecting the scars for inoculation: 1) the leaves were removed mechanically, 2) abscission was induced artificially by severing the leaf blade from the petiole,

thus hastening the subsequent abscission of the petiole, using the technique described by Gawadi and Avery (4), and 3) the scars left by naturally abscising leaves were selected.

In a series of tests using 12 different isolates of the fungus, 20 varieties of *C. japonica*, and 2 varieties of *C. sasanqua*, inoculations were made on the fresh scars of leaves removed mechanically. Moist cotton was wrapped around the inoculated areas and kept moist for 12 hours. Of a total of 1136 such inoculations, 803 resulted in infection. It was noted in these tests that the time elapsing between inoculation and permanent wilting of the shoot distal to the leaf scar was much shorter when the inoculum was applied to the scar of a leaf subtending a lateral shoot than when applied to those located elsewhere on the shoot. The course of the disease resulting from the latter method of inoculation was similar to that produced by the needle prick method. Necrosis of the tissue adjacent to the point of inoculation developed first, and this was followed by wilting and eventual death of the shoot above (Fig. 1). The time elapsing between inoculation and death of the shoot varied greatly, from 4 days to several months. When the inoculum was applied to the scars of leaves subtending young lateral shoots, permanent wilting and death of the shoot occurred rapidly, usually beginning about the fourth day, the average time being 7 days with *C. japonica* var. Prince Eugene Napoleon, and *C. sasanqua* var. Cleopatra. (Fig. 1, E).

In using the deblading technique, plants comprising 6 varieties were placed in a moist chamber and atomized with a conidial suspension. Of 35 shoots with scars of abscised leaves, 14 developed typical dieback (Table 1) and ingress by the fungus in each case occurred through a leaf scar. Check plants similarly treated, except that they were atomized with water instead of a spore suspension, remained healthy.

In another experiment conidial suspensions of 2 separate isolates were applied to scars of naturally abscised leaves. These were fresh scars of leaves which had fallen less than 48 hours prior to inoculation. Moist cotton was wrapped around the inoculated areas and kept moist for about 12 hours. Seven varieties were included, but most of the inoculations were made on the variety Prince Eugene Napoleon. Forty-seven shoots were inoculated, 42 of which became infected and developed typical dieback symptoms. The time elapsing between inoculation and death of the shoot was longer (12–20 days) than in the experiment in which leaves were removed mechanically and inoculum applied to the fresh scar (4–14 days). To account for this difference it may be assumed that the scar tissue of the naturally abscised leaves had become partially suberized and somewhat more resistant to penetration by the fungus.

Of the several techniques used, the most efficient appeared to be the one in which conidia were applied to fresh scars of leaves subtending young lateral shoots. This method was therefore employed in later work in testing the relative susceptibility of different varieties of camellia and also in determining the pathogenicity of different isolates of the Colletotrichum.

The pathogen was reisolated 646 times out of 650 attempts from cankers produced by inoculating fresh scars of mechanically removed leaves.

Varietal tests.—Many varieties of camellias are susceptible to the disease as indicated by results of the inoculation experiments. Nevertheless, it was considered advisable to test the relative susceptibility of a number of varieties by inoculating them using the same time, conditions, and isolate. The pathogenicity of the isolate used had been determined by previous inoculations. Twenty varieties of *C. japonica*, Prince Eugene Napoleon, Mrs. Charles Cobb, Gloire de Nantes, Governor Mouton, Alba Plena, Pink Perfection, Mathotiana, Sarah Frost, Ignescens, T. K. Variegated, Cheerful, La Reine, Caleb Cope, Professor Sargent, Altheaflora, Rubra Virginalis, Dr. Oldwig Thayer, McDaniel Red, Nobilissima, and Flora Plena, and 2 varieties of *C. sasanqua*—Rosea and Cleopatra—were included. The inoculum in each case was applied to the fresh scar of a leaf subtending a lateral shoot. Care was taken to select shoots of approximately the same thickness and age. All varieties became infected. There were, however, differences in degree of susceptibility, indicated by the time that it took the pathogen to kill the shoots. The 2 *C. sasanqua* varieties, Rosea and Cleopatra, were found to be highly susceptible. Inoculated shoots began to wilt and die within 4 days after inoculation, with subsequent canker formation. The *C. japonica* varieties exhibited differences in susceptibility. The varieties Prince Eugene Napoleon, Flora Plena, Nobilissima, T. K. Variegated, and a few others, were as susceptible as the 2 *sasanqua* varieties. At the other extreme, varieties such as Professor Sargent, Governor Mouton, and Ignescens, exhibited a high degree of resistance. The varieties of the latter group became infected, as shown by the discolored, necrotic tissue around the point of inoculation, but most of the inoculated shoots were still alive 1 year after inoculation. The spread of the incipient canker seemed to be checked by the formation of a corky layer. Other varieties, such as Rubra Virginalis and Gloire de Nantes were intermediately susceptible.

Relative pathogenicity of isolates.—In the course of these investigations there were indications that some of the *Colletotrichum* isolates were not pathogenic, or, at least, that there were differences in degree of virulence among different isolates. Eleven isolates of *Colletotrichum* were used to inoculate 2 varieties of *C. japonica*, Sarah Frost and Prince Eugene Napoleon. Twenty young shoots of each variety of approximately the same diameter and the same stage of maturity, were inoculated with each isolate. The needle prick method was used in inoculating the Sarah Frost variety. On the variety Prince Eugene Napoleon, the leaf subtending a lateral shoot was removed mechani-

cally and the inoculum was applied to the leaf scar at the base of the shoot. Results from both inoculation techniques were similar in that 8 of the 11 isolates were pathogenic and 3 nonpathogenic, the same ones being pathogenic on both varieties. In addition to the clear separation into pathogenic and nonpathogenic groups, differences in virulence were noted among isolates, as indicated by the time required for complete wilting of the inoculated shoots. This proof of the existence of nonpathogenic strains (isolates) may account for the failure of previous workers to induce infection by artificial inoculation.

IDENTITY OF THE FUNGUS.—The fungus responsible for dieback and canker in camellias has been designated by previous investigators (1, 2, 8, 9) either as *Gloeosporium* sp. or as *Glomerella cingulata*. It was found during these studies that the fungus produces setae abundantly on cankers and stems and occasionally in culture, indicating that the conidial stage of the causal fungus is a *Colletotrichum* and has been so designated in this paper.

Perithecia with viable ascospores were produced when the fungus was grown on detached camellia leaves in a moist chamber. The characters of this stage were similar to those of *Glomerella cingulata* (Stone) Spauld. and von Schrenk. Mono-ascosporic isolations were obtained and these proved to be identical, culturally and morphologically, with the isolations obtained from dieback cankers and with mono-conidial isolations. These mono-ascosporic isolates proved to be pathogenic on camellia shoots. In inoculations of apple fruit with camellia isolates, typical bitter rot symptoms developed. Isolates of the bitter rot fungus from apple were not available for comparison, but Davis and Pirone (3) reported that the apple fungus was not pathogenic to camellia stems. It is concluded that the fungus causing dieback and canker in camellias should be designated as *Glomerella cingulata* (Stone.) Spauld. and von Schrenck, and its imperfect stage as *Colletotrichum*.

DISCUSSION.—The most significant finding of this investigation, in the opinion of the writers, is the demonstration that infection can take place through the scars resulting from naturally abscising leaves. That this is probably the primary natural avenue of infection is indicated by the fact that in nature the cankers almost invariably occur at the base of lateral shoots, as has been observed by other investigators. Bain (1) in particular stressed the fact that cankers almost always occur at the nodes and suggested that infection starts at the base of the subtending leaf either before or after abscission. The occurrence of dieback approximately coinciding with the shedding of old leaves in the spring furnishes additional evidence that scars serve as points of entry for the pathogen. It is true that dying back of twigs and branches may occur throughout the year, but this does not necessarily imply recent infection. Death of shoots or of larger branches occurs after the cankers have progressed to the stage of girdling. Cankers have been observed to remain dormant or semidormant for long periods.

It would appear logical that if a fungicide were applied to the plants during and just before the period of greatest leaf fall, protection against infection should be attained. Actually, very little experimental work has been done toward the control of this disease by the use of fungicides, and in the few cases where fungicides were tried the results were disappointing. Bain (1) used Bordeaux mixture, ferbam, ziram, and nabam in Mississippi and got no significant control, but this failure could have been due to late application. Bain started his spraying after the first of April when leaf fall had already taken place.

Studies on control of this disease by fungicides which are being carried on at present in this laboratory have not progressed far enough to yield conclusive evidence.

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LITERATURE CITED

1. BAIN, D. C. 1946. Dieback and stem canker of camellia. U. S. Dept. Agr. Pl. Dis. Reptr. **30**: 206-208.
2. DAVIS, B. H. AND P. P. PIRONE. 1941. Anthracnose of camellia. (Abs.) Phytopathology **31**: 6.
3. DAVIS, B. H. AND P. P. PIRONE. 1953. Stem canker of camellia. Sou. Flor. Nurseryman 56 (1): 20-21, 31.
4. GAWADI, A. G. AND G. S. AVERY, JR. 1950. Leaf abscission and the so-called "abscission layer." Amer. Jour. Bot. **37**: 172-180.
5. GILL, D. L. 1938. Camellia wilt and root-rot. Phytopathology **38**: 575-576.
6. KATZ, S. J. 1948. Dieback. American Camellia Yrbk. 120-123.
7. PLAKIDAS, A. G. 1948. Camellia root rot and dieback. La. Agr. Exp. Station Ann. Rept. 1947-1948: 101-103.
8. POST, THELMA B. 1934. Dieback of camellia. U. S. Dept. Agr. Pl. Dis. Reptr. **18**: 112.
9. WEST, E. 1946. Camellia dieback. Amer. Camellia Yrbk. 46-48.
10. WEST, E. 1947. Camellia diseases. Amer. Camellia Yrbk. 116-121.

A NEW FRUIT ROT OF PEACH [1]

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SUMMARY

An undescribed fungus was found to be parasitic on peaches in Louisiana, and the name *Diplodina persicae* n. sp. was assigned to it.

All varieties of peaches tested were found to be susceptible when inoculated under artificial conditions. Southland and Burbank Early Elberta were the most susceptible whereas Sunhigh was the least susceptible.

The organism fruited and produced the greatest mycelial development on lima bean and potato-dextrose agars. Optimum temperature for growth and production of pycnidia was 27° C. The addition of 1, 2, or 3 drops of 25 per cent lactic acid to 5 cc. of medium greatly reduced the growth of the fungus.

Captan, ferbam, and Craig Experimental Fungicide 5400 were effective in preventing fruit rot in laboratory tests.

During the harvesting season of 1952 and 1953 an undescribed fruit rot of peaches was observed in Louisiana orchards. The causal organism was tentatively identified as *Diplodina* sp.[2] Early symptoms of the disease were similar to those produced by the brown rot organism *Sclerotinia fructicola* (Wint.) Rehm.; until the present investigation was made, growers assumed that *S. fructicola* was the only cause of the brown rotting of fruit. Since the organism was found widely distributed over the peach growing areas of Louisiana and its pathogenicity demonstrated in the laboratory, the disease could be potentially of economic importance.

SYMPTOMS.—*Diplodina* sp. was isolated from young twigs, fruit, and leaves. Symptoms on fruit were described and illustrated in a previous report.[2] When young twigs and leaves were artificially inoculated and incubated under conditions of high humidity in the greenhouse, they became severely infected (Fig. 1, E). The spots were mostly irregular in pattern, sometimes oval to round, up to 15 mm. in diameter, and frequently formed alternating brown to black concentric rings. Pycnidia, when present, were scattered over the infected areas. Naturally-infected leaves were similar in appearance. On the inoculated twigs chocolate colored, somewhat sunken areas developed which later became dark brown. Approximately 2 weeks after inoculation pycnidia were found in abundance scattered over these infected surfaces.

PHYSIOLOGICAL CHARACTERISTICS.—The growth of the organism was tested on various agar media, including potato-dextrose, yellow cornmeal, lima bean, navy bean, Czapek's, dextrose, and oatmeal agars. Growth was determined by increase in diameter of the colonies on the above-mentioned agars. Uniform discs of mycelium 3 mm in diameter, cut from agar plates were used for the inoculum. All the media except yellow cornmeal and dextrose agars were favorable for the growth of the fungus. Oatmeal was the most suitable medium for mycelial development, but conidia were seldom produced on this medium. Instead, small scattered sclerotial masses were formed. Lima bean and potato-dextrose agars were the most suitable media for the growth of mycelium and for the development of pycnidia. Growth determinations at various temperatures were made by the following procedure: inoculum discs of mycelium were transferred to 125 ml. flasks containing sterilized 2 per cent dextrose and 2 per cent yeast extract solution; 4 flasks of each were placed in controlled temperature chambers of 13°, 18°, 25° 27°, 31°, 35°, and 40° C. for a period of 5 days; the contents of the flasks were filtered under pressure through No. 9 filter paper discs seated in Gouch crucibles; the mycelial mats were dried at 90° C. for 2 hours and weighed. The greatest amount of growth was obtained at 27° C. No measurable growth occurred at 13° or 40° C.

The fungus was grown at room temperature (24–35° C.) in potato-dextrose medium to which were added varying amounts of 25 per cent lactic acid. One drop of acid in 5 cc. of medium reduced the growth nearly ½, 2 drops approximately to ⅛, and 3 drops almost completely stopped growth.

PATHOGENICITY STUDIES AND VARIETAL SUSCEPTIBILITY.—Preliminary tests to determine the pathogenicity of the organism were made. Fruit was inoculated with the fungus by dipping into a conidial suspension. In 1 series the fruit was punctured with a sterile needle several times before inoculation. Non-inoculated fruits were used as controls. After treatment all the fruit was placed in moist chambers. Within 24 hours spots appeared on the treated peaches. The injured fruit contained spots not only at the wounded areas but at other locations as well. Approximately 5 days later the inoculated fruits were almost entirely rotted.

Leaves and stems were also inoculated with spore suspensions of the fungus. The cuttings were placed in tap water under a cover similar to a bell jar in which high relative humidity was maintained. After 4 days many leaves were severely infected.

Six varieties of peaches were tested for resistance. Two means of inoculation were used; in one, the

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[2] Horn, N. L. 1952. *Diplodina* on peach in Louisiana. Plant Dis. Reptr. 36: 351.

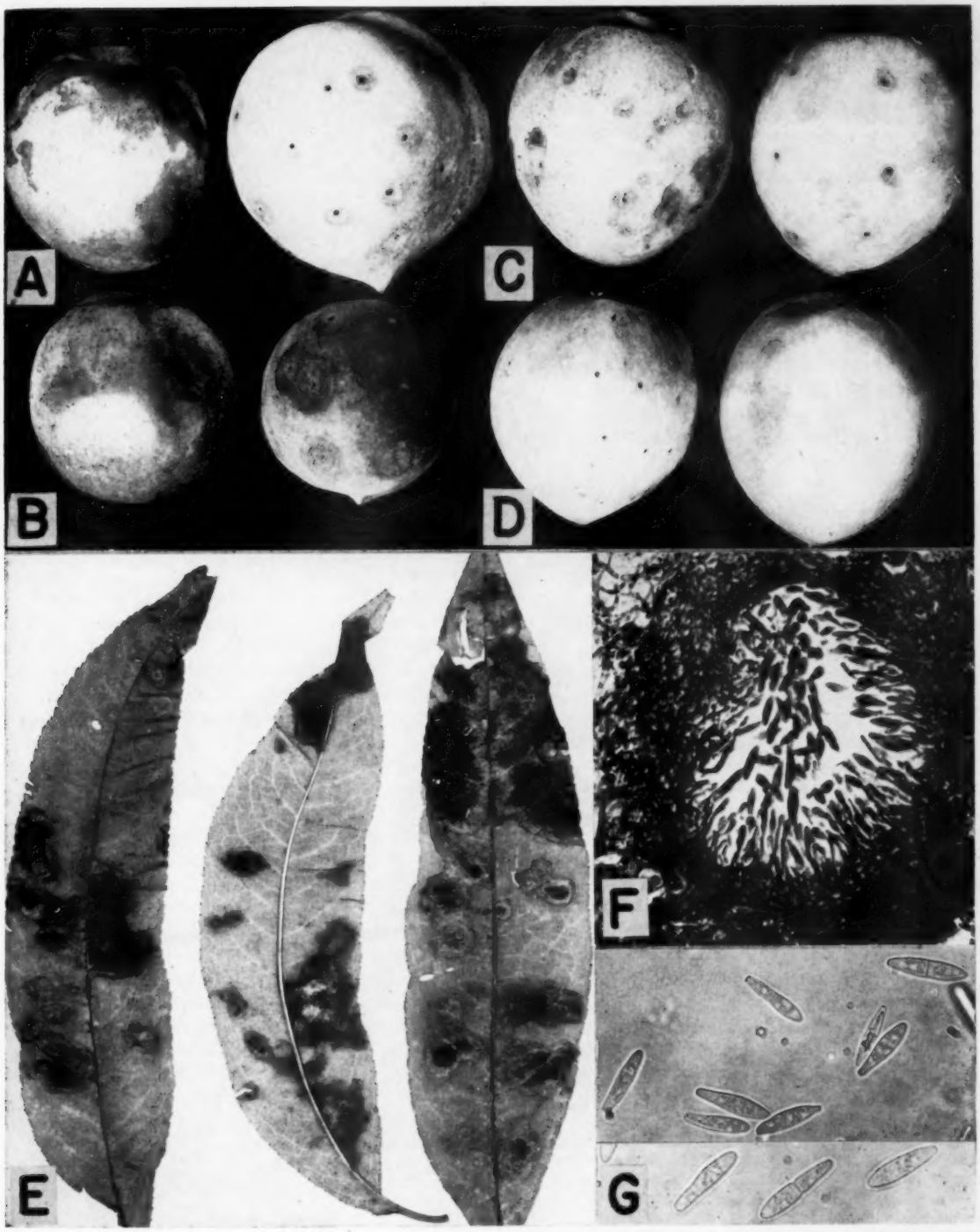

FIG. 1. A-C were fruits inoculated with *Diplodina* sp., one peach of each series was dipped into a conidial suspension, the other jabbed with an infested needle; A) Halehaven; B) Southland; C) Sunhigh; D) Sunhigh was used for control. E) Inoculated leaves. F) Pycnidium (photographed from a stained mount), and G) Conidia of *Diplodina* sp.

peaches were dipped in a conidial suspension, then incubated in moist chambers for 3 days at room temperature (24–35° C.); in the other, the fruits were punctured with a needle which had been dipped in a conidial suspension before inoculation (Fig. 1, A-C). Controls consisted of noninoculated fruits, some of which were jabbed with a sterile needle (Fig. 1, D). Three fruits were used for each treatment. Symptoms appeared 24 hours after inoculation on both punctured and uninjured fruit. All varieties became in-

fected, but some were more susceptible than others. The following varieties are listed in decreasing susceptibility: Southland, Burbank Early Elberta, Halehaven, Triogem, Southaven, and Sunhigh.

The susceptibility of each variety was the same irrespective of method of inoculation used, except Southland and Triogem. The former appeared more susceptible when the puncture method was used, and Triogem seemed more susceptible when inoculated by the dip method. Sunhigh appeared to be the least susceptible with both methods. In previous tests, Dixigem was found to be highly susceptible to *Diplodina* sp. Spots up to 18 mm. in diameter were observed on fruit 3 days after inoculation.

The organism: *Diplodina persicae* n. sp.[3]—The fruiting body a pycnidium, dark colored, ostiolate, glabrous, spherical, erumpent, discrete, average size 175 μ (range 130 μ–210 μ); conidiophores simple up to 25 $\mu \times 2.5$ μ; conidia 2-celled, infrequently 3-celled, unequal in size, the terminal end tapered to a blunt point, the basal end flattened, hyaline (mass color cream-buff[4]), frequently constricted at the septum, average size 26.5 $\mu \times 6.5$ μ (range 22 μ–30 $\mu \times 5.5$ μ–7.0 μ).

Habitat: Parasitic on fruit, stems, and leaves of *Prunus persica* (L.) Batsch. in Louisiana.

Specimens have been filed in the herbaria of the Department of Botany, Bacteriology and Plant Pathology, Louisiana State University, Baton Rouge, Louisiana; Mycological Collections, Bureau of Plant Industry, Beltsville, Maryland, and the Department of Plant Pathology, Cornell University, Ithaca, New York. Collections were made on *Prunus persica* (L.) Batsch., June 23, 1953, by N. L. Horn at the North Louisiana Hill Farm Experiment Station, Homer, Louisiana. No. CUP 44419, Dept. Plant Path., Cornell University, is designated as type.

CHEMICAL CONTROL.—A series of experiments were made in an attempt to find a chemical control for the disease. Two methods were used. In one case conidial germination tests were made on chemically treated glass slides. In the other the degree of infection was determined after the fruits were dipped in combined suspensions of conidia and fungicides and then incubated.[6] Seven fungicides were tested: captan, the salt of dehydro acetic acid (DHA-S), Craig Experimental Fungicide 5400, zineb, triBasic copper sulfate, ferbam, and sulfur. Each was used at the rate of 4 lbs. per 100 gal. of water.

TABLE 1.—*The germination of conidia in potato-dextrose medium placed on chemically treated slides*

Treatment	Number Counted	Number Germinated	Remarks
Control	200	200	formed mycelial mat after 18 hours
Captan	200	0	cells vacuolate
DHA-S	200	200	formed mycelial mat
Craig 5400	200	0	cells vacuolate
Zineb	200	106	germ tubes up to 5 $\times$ the length of the conidia
TriBasic $CuSO_4$	200	0	cells appeared normal
Ferbam	200	0	cells vacuolate
Sulfur	200	133	germ tubes up to 5 $\times$ the length of conidia

In the former method germination counts were recorded after 18 hours. Because of the difficulty in obtaining quick germination in tap water or distilled water, potato extractions and dextrose were added. The results of the germination tests are in Table 1.

The conidia were examined 24 and 48 hours later, but still there was no germination in treatments with captan, 5400, ferbam, or triBasic copper sulfate.

The best treatments by the slide method were also the most effective by the fruit dip method with the exception of triBasic copper sulfate. Fruit treated with ferbam, captan, and Craig Experimental Fungicide 5400 were not infected at the end of 4 days. The controls consisted of untreated fruit and fruit that was dipped in a conidial suspension. Fruits used in the latter control were severely rotted. Results were the same in 3 tests in which Fay Elberta, Dixigem, and True Early Sullivan Elberta, respectively, were used. Although sulfur is used extensively for the control of brown rot (*Sclerotinia fructicola*), it was very ineffective against *Diplodina* sp. under the conditions tested in the laboratory.

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[3] The description of *Diplodina arenariae* Massee, Kew Bul.[5] reported on *Arenaria vernae* from Red Mount, Spitzbergen, resembles closely the *Diplodina* specimens presented here. The greatest dissimilarity was the size of the pycnidia; those of *D. arenariae* were up to 4 $\times$ the diameter of the pycnidia described in this paper. *D. pruni* Ell. and Barth. was reported on *Prunus demissa*, which is native to Northwest United States, but the organism described here does not fit the description of that species.

[4] Ridgeway, R. 1912. Color Standards and Color Nomenclature. Washington, D. C. Published by author.

[5] Saccardo, P. A. 1899. Sylloge Fungorum 14: 936.

[6] Horn, N. L. 1952. Strawberry fungicide screening tests. Plant Dis. Reptr. 36: 309-310.

RELATION OF STAGONOSPORA, RHIZOCTONIA, AND ASSOCIATED FUNGI TO CROWN ROT OF ALFALFA [1]

Donald C. Erwin [2]

SUMMARY

Three crown and root diseases of alfalfa have been investigated. These are: a dark crown necrosis, which could not be reproduced by any of the fungi commonly associated with it; Stagonospora crown and root rot incited by *Stagonospora meliloti;* and Rhizoctonia stem and crown canker, by *Rhizoctonia solani.*

Dark, necrotic crown rot, the most prevalent crown disease of alfalfa, consists of longitudinal or wedge-shaped lesions on crowns and crown branches. Fungi from diseased plants, the majority of which were *Fusaria,* were tested on healthy plants, but none reproduced the disease.

Experiments in which inoculum of *S. meliloti* was placed over alfalfa seeds have shown that seedling infection may occur without wounding. Although no method of inoculation induced 100 per cent infection consistently, dipping roots and crowns in a spore or mycelial suspension for 30 minutes and later incubating in a moist chamber for 2 days prior to transplanting, produced high percentages (87–100 per cent) of infection in the variety Africa which contained more susceptible plants than the variety California Common. In more limited trials the Atlantic variety of alfalfa contained the least number of susceptible plants.

Stagonospora inoculum did not survive after 3 months in sterilized or non-sterilized soil at a level sufficient to induce disease.

Radial growth of *Stagonospora meliloti* on bacto-peptone agar was greatest at 18° C. Growth of aerial mycelium and formation of pycnidia on steam-sterilized alfalfa stems was greatest at 18° C. Growth of mycelium and formation of pycnidia occurred also at 5° C. Earliest germination of pycnidiospores occurred at 21° to 27° C. in 1 hour and 45 minutes. Spores eventually germinated at 5° but not at 33° or 36° C.

Stem and crown bud cankers were caused by *Rhizoctonia solani. R. solani,* was shown to be in the vessels of infected stems, and caused a discoloration of the crown tissue above and below the canker.

Of 24 isolates of *Rhizoctonia,* which showed pathogenic as well as cultural differences, twelve were pathogenic to crown stems and seedlings, 1 was pathogenic only to seedlings, and 10 were non-pathogenic.

Crown rot diseases of alfalfa are found in almost any irrigated field in California. Their importance is probably next to that of bacterial wilt, caused by *Corynebacterium insidiosum* (McCull.) H. L. Jens. and alfalfa dwarf caused by the Pierce's disease virus. No one description can account for all the types and manifestations. Three crown and root diseases of alfalfa have been investigated. These are: dark crown necrosis, which could not be reproduced by any of the fungi associated with it; Stagonospora crown and root rot incited by *Stagonospora meliloti* (Lasch.) Petr.; and Rhizoctonia stem and crown canker by *Rhizoctonia solani* Kühn.

Most frequently dry necrotic, dark brown to black, tissue is found on the crowns and crown branches of plants with dark crown necrosis. The affected areas may be wedge-shaped in the center of the crown or lesions may appear longitudinally on one side of a crown branch. Eventually the affected crown branches die and in severe cases older plants are killed. The disease is designated in this paper as dark crown necrosis and its cause is not known. A search for a single causal agent has failed. Results of extensive isolations and pathogenicity tests conducted with isolates from plants with this crown rot are given.

Since *Stagonospora meliloti* has been reported as an incitant of alfalfa root rot (18) and occurs in California, this fungus was studied to assess its importance in relation to crown rot, to determine the best method of inoculation, the cultural relations of the fungus, and the varietal reactions of Africa, California Common, and Atlantic alfalfa to *Stagonospora.* Symptoms of Rhizoctonia crown bud necrosis and stem canker caused by *Rhizoctonia solani* Kühn and the variation in pathogenicity among several isolates of this fungus is also described.

LITERATURE REVIEW.—Fusarium root rot of alfalfa was reported by Brown and Gibson (3), Melchers (20), and others, but experimental proof of the pathogenicity of the *Fusaria* was not presented. Pathogenicity of *Fusarium* on seedlings has been reported (9). Weimer (28), after an extensive survey of eastern and central United States, described collar rot, heart rot, hollow crown, and a decay of the tap roots about 4–6 in. below the surface of the soil. Different species of *Fusarium,* bacteria, and *Rhizoctonia* were isolated but these organisms were not capable of reproducing the diseases. In a later report of Fusarium wilt, caused by *Fusarium oxysporum* f. *medicaginis* (Weimer) Sny. and Han., Weimer (29) again noted that there were many reports of Fusarium root rot, but experimental proof of the pathogenicity of *Fusarium* on alfalfa plants beyond the seedling stage was lacking. Later Cormack (6), working in Canada, showed that *Fusarium avenaceum* (Fr.) Sacc., *F.*

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arthrosporoides Sherb., *F. culmorum* (W. G. Sm.) Sacc., *F. scirpi* Lamb. et Fautr. var. *acuminatum* (Ell. et Ev.) Wr., and *F. poae* (Peck) Wr. were causal agents of root and crown rot. Staten and Leyendecker (27) proved the pathogenicity of *Fusarium solani* (Mart.) Appel and Wr. on alfalfa roots in New Mexico. Jones and Weimer (18) reported that *Stagonospora meliloti* (Lasch.) Petr. caused a root and crown rot of alfalfa. Briton-Jones (1) showed that *Rhizoctonia solani* Kühn from cotton was also pathogenic to alfalfa. Crown bud necrosis caused by *Rhizoctonia solani* has been reported (8, 12, 14). Cherewick (4) stated that *R. solani* was probably the sole cause of crown rot of alfalfa and sweet clover in Manitoba. *R. solani* also causes a root canker disease which is favored by a high soil temperature (25, 26). *Cylindrocarpon ehrenbergi* Wr. (5), *Ascochyta imperfecta* Peck (*Phoma medicaginis* Malbr. and Roum.) (7, 16, 21, 22), *Diplodia gossypina* Cke. (24), *Plenodomus meliloti* D. and S. (23), *Rosellinia necatrix* (Hart.) Berl. (13), and an unidentified basidiomycete (2), have all been shown to be pathogenic on roots or crowns of alfalfa. Alfalfa is also susceptible to *Colletotrichum trifolii* Bain and Essary (17).

DARK CROWN NECROSIS OF ALFALFA.—Isolations were made from margins of necrotic tissue from plants with various types of crown rot. Over 40 total isolation experiments were conducted and isolations made from at least 5 to 10 plants per experiment. Plants were mostly selected from alfalfa fields near Davis, California; however, fungi from some plants grown in Modoc County, the Imperial Valley, and the San Joaquin Valley were also isolated and tested.

No attempt to estimate quantitatively the frequency of occurrence of fungi was made; however, *Fusarium roseum* was isolated most often, followed by *F. solani*. *Coniothyrium* sp., a pycnidial fungus, was also associated with diseased crown and root tissue in many plants. Occasionally *Rhizoctonia solani* was isolated from a diseased stem. *Pythium* spp. were sometimes isolated from crown buds, crowns, and lateral roots.

Forty-one isolates of *Fusarium*, (26 *F. roseum* and 15 *F. solani*), 35 pycnidial fungi, and 5 sterile mycelium types were tested for pathogenicity in the greenhouse. The methods consisted of either pouring a spore suspension over the seed in a row (fungi tested this way were also tested with 1 of the following methods), pouring a spore suspension over wounded crowns, placing mycelium and agar on wounded crowns, dipping roots of plants in a spore suspension prior to transplanting, or transplanting to soil infested with an oat culture of the fungus. In all tests at least 20 plants were inoculated with each isolate and adequate checks maintained for each experiment. All inoculation methods were considered to be severe enough to detect a pathogen. Plants were grown in soil which had been sterilized with steam at 15 lbs. pressure for 3 hours.

Three *Fusarium roseum* isolates, 1 *Phoma* sp. and several *Pythium* spp. were pathogenic only when inoculum was placed on seed; however, none of the fungi tested caused a crown or root rot on 1–4-month-old plants. Two of the *Fusarium roseum* isolates caused a damping off of seedlings similar to that commonly caused by *Rhizoctonia solani*. However, virulence was not great since less than 50 per cent of the plants damped off in 11 days after inoculation. The other *Fusarium roseum* isolate, morphologically similar to the fungus designated by Cormack (6) as *F. avenaceum* (Fr.) Sacc. and obtained from a diseased crown bud, caused 100 per cent damping off of emerging seedlings but was not pathogenic to plants beyond the seedling stage. *Phoma*-infected seedlings became black about ½ in. above the soil line and then toppled over. The *Phoma* sp. caused over 50 per cent seedling loss after 11 days when soil temperature was less than 25° C. When temperatures were high (over 25° C.), little damping off occurred, and some seedlings that showed the black stem condition recovered. The *Pythium* spp. caused nearly 100 per cent seedling loss.

In addition to the greenhouse tests a field test was set up in which 2-month-old plants of Africa, California Common, Briggs Common, and Hilmar varieties of alfalfa were inoculated in the spring with ground oat inoculum of the California *Fusarium roseum* isolate which had caused 100 per cent seedling loss in greenhouse tests. Samples (150 to 200 plants per plot) were examined during July of 1952 and in June of 1953 and found to be free of disease. A like test was conducted with the *Coniothyrium* sp. and likewise no crown or root rot was found.

In a further attempt to isolate the pathogen causing dark crown necrosis, diseased crowns were cut up and incorporated into steamed soil. Alfalfa seeds were then planted in the soil or large plants, grown in steamed soil, were transplanted to this soil. The roots of these plants were removed after 2 months and examined. Occasionally some plants were infected by *Pythium* spp. or *Rhizoctonia solani;* however the symptoms of the original dark crown necrosis were never produced.

Although negative evidence should be viewed with caution, there was no indication that the *Fusarium* fungi and other organisms closely associated with crown rot were pathogenic on alfalfa plants beyond the seedling stage in these tests. There is the possibility that in the field there may be a predisposing condition which allows weak parasites to attack the plants. Since evidence of pathogenicity of the most frequently isolated fungi was lacking, the possible relationship of *Stagonospora meliloti* and *Rhizoctonia solani* to crown rot was studied.

STAGONOSPORA CROWN AND ROOT ROT.—The occurrence of this disease caused by *Stagonospora meliloti* was somewhat sporadic during this study. In 1951 it was seldom seen whereas in 1952 in the same fields the disease was found frequently as early as January. Although the fungus may be lethal to infected plants and in some years may be important, it is not the sole

cause of crown rot in California. However, since it has been noted frequently in the state, a study of the effect of environment on the fungus in culture and its pathogenicity on several varieties of alfalfa was initiated.

Symptoms of Stagonospora root rot.—The most distinguishing symptom of the disease is the reddish flecking in the bark and wood of an infected crown branch or root (Fig. 1). This symptom serves to distinguish this disease from dark crown necrosis. Necrosis of infected tissue is not evident in early stages and since plants with large infected areas on the tap roots seldom show any stunting or other top symptoms, much of the tissue is probably functional. The bark of the infected roots often cracks and becomes rough. Eventually the reddish tissue becomes necrotic and rotten, and death of the infected crown branch or root follows.

Stagonospora also causes a leaf spot disease of alfalfa and sweet clover (15, 18). However, this phase of the disease is not important in California. Leaf spots, caused by *Stagonospora*, were induced artificially in a greenhouse moist chamber by spraying leaves with a pycnidiospore suspension. Oval, necrotic spots, which sometimes showed concentric rings, occurred about 2 weeks after inoculation. Pycnidia of the fungus formed on the spots 1–2 weeks later. Necrosis of stems and petioles also occurred in these tests. Symptoms were similar to those previously described (15, 18).

Pathogenicity of Stagonospora meliloti.—The fungus grew very slowly in culture and consequently was difficult to isolate. Isolation was accomplished only when a piece of infected tissue to be plated was free of other organisms. Potato dextrose agar (hereafter designated as PDA) at a pH of 4.0 to 4.5 was used successfully as a substrate.

A method resulting in a high percentage of disease incidence and by which large numbers of plants could be inoculated quickly was sought. Placing inoculum in a wound and covering with cotton or tape was successful in most cases, but was too slow for possible use in a plant breeding program. In 6 tests when oat inoculum was placed on seed planted in sterilized soil, disease incidence on the most susceptible variety, Africa, ranged from 20 to 66 per cent with an average of 43. These data showed that perhaps many plants escaped infection.

In 3 subsequent experiments 92 Africa plants (about 2 months old) were inoculated by dipping roots in a viable spore suspension for at least 30 minutes and incubating in a moist chamber for 2 days prior to transplanting to soil. The disease incidence ranged from 87 to 100 per cent with an average of 93 per cent. This inoculation method was the most successful. Apparently infection occurred more readily in an environment away from the soil. Field observations have indicated that this parasite probably enters the plant through the above-ground crown branches and progresses downward in the bark and woody tissues to the tap root.

FIG. 1. Alfalfa plant naturally infected by *Stagonospora meliloti* collected in field near Davis, California. Enlarged longisection of the crown shows the flecking of the woody tissue which naturally appears red.

The fungus did not produce symptoms on plants in the seedling stage. When seed was inoculated by pouring large volumes of a spore suspension over seed, the percentage of diseased plants 3 months after inoculation ranged from 13 to 26 with an average of 20. A total of 478 plants in 5 experiments was inoculated in this way. Evidence of disease on roots was not usually present until plants were about 2–3 months of age. The apparent lack of symptoms on seedlings was probably due to the slow growth of the fungus after infection had occurred rather than to resistance of the young plant.

Fifteen days after the seeds had been inoculated by placing spore or oat inoculum over them, small plants were dug, washed, and surface sterilized for 3 minutes with a 0.5 per cent solution of sodium hypochlorite. They were then re-washed in sterile distilled water and plated on acid PDA. Seven days later *Stagonospora* colonies appeared on 13 per cent of the seedlings. This was evidence that infection probably had occurred in the small plant roots. In several other ex-

TABLE 1.—*Comparison of the disease incidence in alfalfa plants inoculated with oat inoculum of Stagonospora in steamed and non-steamed soil*

Experiment	Soil	Days from inoculation to reading No.	Variety	Plants inoculated No.	Disease Incidence Per Cent
1	Steamed	131	Africa	137	66
	Non-steamed	131	Africa	42	24
	Steamed	131	Calif. Common	208	52
	Non-steamed	131	Calif. Common	46	6
1b	Steamed	106	Africa	240	20
	Non-steamed	106	Africa	165	3
	Steamed	106	Calif. Common	246	12
	Non-steamed	106	Calif. Common	176	7
	Steamed	106	Atlantic	304	4
	Non-steamed	106	Atlantic	302	2

periments in which 16 to 39 days had elapsed between inoculation and plating, the percentage of plants containing *Stagonospora* varied from 16 to 100. However, no symptoms were evident on the roots. These experiments showed that infection occurred in young seedlings as early as 15 days after inoculation, but that initiation of visible symptoms was delayed until later than 5 weeks after inoculation.

It was noted in early pathogenicity tests that the fungus did not remain viable in the soil from 1 test to the next. This was further tested by replanting Africa alfalfa seed in soil which had been infested with an oat culture of the fungus 3 months previously and from which diseased roots had been removed. In no case did plants growing from this soil become diseased.

To test the virulence of *Stagonospora meliloti* in unsterilized soil, seed of California Common, Africa, and Atlantic varieties were inoculated by placing a large quantity of oat inoculum on top of the seeds in sterilized and unsterilized soil. The experiment was repeated once. In both experiments the disease incidence was much less in non-sterilized soil than in sterilized soil (Table 1). It seemed possible that the soil microflora may have suppressed the slow-growing pathogen before infection occurred. This fungus is probably a root-infecting fungus of the root-inhabitant type (10).

Stagonospora meliloti was isolated several times from necrotic leaf bracts and stems. Although leaf and stem infection does not occur abundantly enough in California to be damaging to foliage. this phase of the disease is probably important since pycnidia, forming in the necrotic areas, are a source of pycnidiospore inoculum that may be disseminated in irrigation water or by transportation of diseased leaves or stems. Pycnidia were not found on infected root tissue. Since the fungus did not exist in infested soil at a high enough level to incite disease, *Stagonospora* is probably dependent upon above-ground pycnidia as a source of primary inoculum. Jones and Weimer (18) reported that the perithecial stage of the fungus (*Leptosphaeria pratensis* Sacc.) was found occasionally in the spring. Perithecia were not found during this study.

Varietal reaction to Stagonospora meliloti.—Jones and Weimer (18) noted that the Peruvian and non-hardy varieties of alfalfa were more susceptible to foliage infection than hardy Turkestan plants. Hanson (11) rated Africa the most susceptible to natural infection of foliage in the field.

Eight separate tests of the pathogenicity of *Stagonospora* on the roots of Africa and California Common alfalfa were conducted. In some tests the variety Atlantic was included. Africa contained more susceptible plants than did California Common or any other variety tested. Conversely Atlantic, though not tested so often, consistently contained the smallest number of susceptible plants. The data indicated that there were varietal differences, but the presence of a large number of escapes made interpretation of the data difficult. For instance, in 1 test only 59 per cent of the plants, originally inoculated by placing oat inoculum over the seed, showed symptoms. Thirty-one of the non-diseased plants were reinoculated by dipping the roots in a spore suspension and holding the plants in a moist chamber for 2 days prior to transplanting. Sixty-eight per cent of these plants showed symptoms after 3 months. In another test, in which the plants had been inoculated by the root dipping method just described, 80 per cent became diseased. The remaining 20 per cent were reinoculated, using the same method, and nearly half these plants showed symptoms 3 months later. These data indicated that a large percentage of the non-diseased plants in each varietal test probably escaped infection.

Growth of Stagonospora meliloti in culture.—Isolates from alfalfa grew very slowly on all media used. On PDA the mycelium was mostly superficial and a thick black stroma formed near the initial inoculum piece. Older colonies often became convolute and raised at the center of the plate. The margins of a colony were usually irregular, and the lobed areas often extended considerably farther in one direction than another. Quite often this growth became slightly submerged and the fungus stroma did not form. The fungus seldom reached the edge of the agar substrate

and commonly caused PDA to turn dark brown after 2–3 weeks. This indicated that perhaps a staling product, which may be inhibiting or retarding to growth, was formed. Pycnidia were produced in a dark stroma on PDA after 20–30 days and separately, but not in a stroma, on alfalfa stems in 8–10 days. Pycnidiospores were 1- to 2-celled, occasionally 3-celled, but mostly 2-celled.

Since growth on PDA was so slow and margins so irregular, a medium was sought which would support a more rapid growth. A nutrient agar that favored the production of more symmetrical and larger colonies was obtained by substituting Difco bacto-peptone for NH_4NO_3 in a nutrient medium[3] devised by Dr. B. R. Houston. Colonies on this agar were uniform and smooth and showed less of the variability that occurred so readily on PDA. No growth occurred on Houston's bacto-peptone medium when NH_4NO_3 was used in place of bacto-peptone as the nitrogen source. Pycnidia were not formed on bacto-peptone nutrient agar. After an incubation period of 18 days the average diameter of colonies on PDA was 18 mm., while on bacto-peptone nutrient medium it was 29 mm.

Pycnidiospores from apparently normal cultures often were not viable. This was especially true of those obtained from cultures 6–8 weeks old on PDA. However, some lots of spores obtained from young cultures on alfalfa stems also were non-viable. Germ tubes usually appeared on viable spores in 1½–2 hours after the spores were placed in a water drop.

Effect of temperature on growth of the fungus.—Variation in temperatures used for the following experiments was less than 1° C. The effect of temperature was determined by measuring radial growth on bacto-peptone nutrient agar, recording percentage of germination of pycnidiospores, and rating the growth and formation of pycnidia on sterile alfalfa stems at each temperature. In the radial growth experiments the initial inoculum consisted of a 4 mm. disc of agar which had been flooded with a spore suspension and incubated for 24 hours. The inoculum disc was placed (mycelium side down) in the center of a petri dish containing approximately 20 ml. of agar. Five plates were incubated at each temperature and the experiment repeated 4 times. Measurements of the growth of the fungus were made in 9–15 days, and variation in the diameter of the colonies at each temperature was not greater than 1 mm. per colony. The optimum temperature for radial growth was 18° C. (Fig. 2). The isolate 97 consistently grew more rapidly than 28, but both had the same temperature optimum.

Spores, obtained from pycnidia on inoculated, steam-sterilized alfalfa stems, were used to determine the effect of temperature on germination. The concentration of spores was adjusted to approximately 50 spores per field of a microscope equipped with 10× oculars

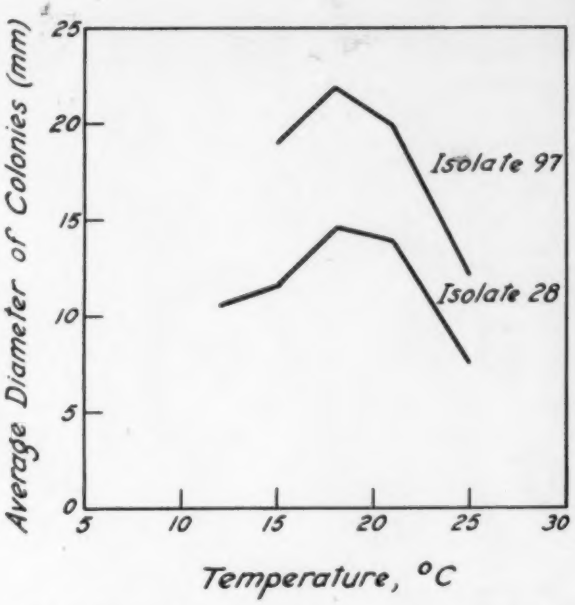

FIG. 2. Effect of temperature on the radial growth of 2 isolates of *Stagonospora meliloti* on bacto-peptone nutrient agar after 14 days.

and a 10× objective and enough bacto-peptone added to the spore suspension to make a 0.1 per cent solution. Four drops of this spore suspension were placed on each chemically clean slide which was then placed in a moist chamber. Slides as well as moist chambers were preincubated at each temperature for at least 12 hours. A count of the number of germinated and non-germinated spores in 1 or 2 microscopic fields of each drop was made as soon as the earliest germination could be detected. The data were compiled from 6 experiments in which a total of 400–500 spores were counted at each temperature.

Germination of spores began as early as 1½ hours after they were placed in the dilute peptone solution, and readings were taken in 1¾ to 2¼ hours at 5°, 12°, 19°, 21°, 24°, 27°, 29°, 33°, and 36° C.

Most rapid germination occurred between 21° and 27° C., but at 19° and 29° the percentage of germination and length of germ tubes were much less. Spores germinated well at 5° and 12° after 10–11 hours, but failed to germinate at 33° or 35° C. Germ tube length was always greatest at 24° to 27° C.

Stagonospora meliloti grew well on alfalfa stems and at room temperature (20 to 25° C.) formed pycnidia in 8–10 days. To determine the temperature at which pycnidia formed the most readily and at which the greatest amount of visible, aerial growth occurred, 4 alfalfa stems, approximately 2 in. in length, were placed in each test tube, steam-sterilized, inoculated with 2.5 ml. of a spore suspension and placed at 5°, 12°, 15°, 18°, 21°, 25°, and 30° C. In this experiment, which was repeated once, 6 tubes of stems were placed in each moist chamber. All tubes were then placed at 18° C. for 1 day to insure adequate spore germination

[3] Bacto-peptone 10 gm., K_2HPO_4 1.35 gm., KH_2PO_4 0.15 gm., KCl 0.15 gm., $MgSO_4$ 0.75 gm., $FeCl_3 \cdot 6H_2O$ 0.002 gm., glucose 20 gm., agar 20 gm., distilled H_2O to make a total of 1 liter.

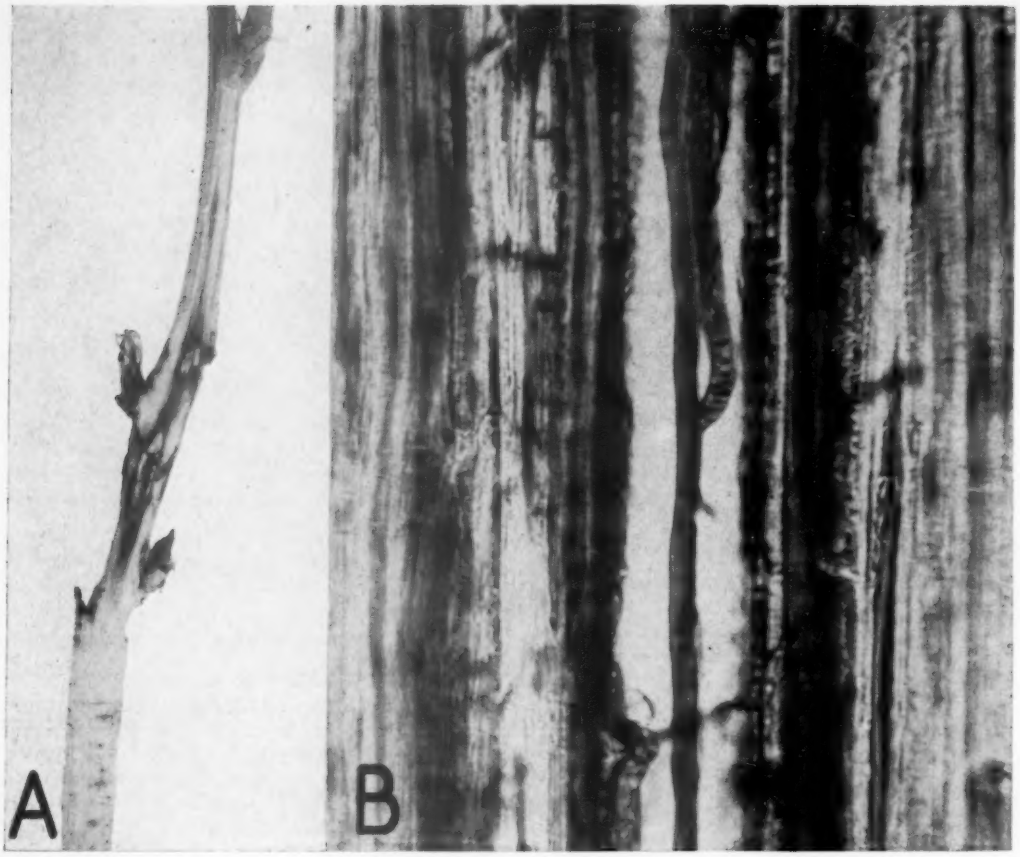

FIG. 3. Stem canker and crown bud necrosis caused by inoculation of alfalfa in the field with *Rhizoctonia solani*. A) Stem was split to show the discoloration in the wood above and below the 2 cankers located near the left lateral bud. B) Mycelium of *R. solani* in a vessel 1.5 cm. above a stem lesion. Darkened regions on both sides of invaded vessels do not contain the fungus. (×400)

after which 1 moist chamber was placed at each temperature for 21 days.

Aerial growth of mycelium in all tubes at 12°, 15°, and 19° C. was cottony and abundant with the most growth at 19° C. At 25°, 27°, and 30° C. mycelium was not cottony but of a hard, stromatic consistency. Mycelium was sparse at 5° C. but some growth occurred. Pycnidia formed at all temperatures, but were much more abundant at 12°, 15°, and 19°, with 19° C. appearing to be the optimum for pycnidia formation.

In this study 15° to 19° C. appeared to be optimum for radial growth on agar and mycelial growth on stems, whereas, the optimum for spore germination was at a higher temperature range (21° to 27° C.).

CROWN BUD NECROSIS AND STEM CANKER INCITED BY RHIZOCTONIA SOLANI KUEHN.—Two manifestations of disease incited by *Rhizoctonia* were investigated, crown bud necrosis and stem canker. Crown bud necrosis has been found throughout the year. Stem cankers are formed below the soil line on crown branches.

Symptoms of the disease.—Crown bud necrosis of the infected buds or shoots usually followed soon after the appearance of water soaking, and these shoots were invariably killed whenever attacked. Isolation from diseased buds yielded both parasitic and nonparasitic *Rhizoctonia* cultures as well as several *Fusarium* and *Pythium* spp. Crown bud necrosis was reproduced in sterilized soil by some isolates of *Rhizoctonia solani* and *Pythium* spp., but not by any of the *Fusarium* spp. isolated.

The stem cankers were usually delimited and dark and nearly always completely girdled the stem causing its death. Browning of the woody tissue above and below the lesions on stems was noted. The browning often extended into the crown area and sometimes resembled the dark crown necrosis described in the first section.

Pathogenicity of various isolates of Rhizoctonia solani.—Since the literature on variation of *Rhizoctonia solani* is voluminous and has been reviewed recently by Kernkamp and co-workers (19), no attempt will be made to cite all papers concerned with the variation of *R. solani*.

Rhizoctonia solani was isolated from plants with root canker, stem canker, and crown bud necrosis. Of 24 hyphal tip isolates tested only 12 were pathogenic to alfalfa crown stems and seedlings. 1 isolate was

pathogenic only to seedlings but not to crowns or roots of 1-month old plants, and 10 were non-pathogenic even to seedlings. Root cankers in this test were not severe and the fungus was most active on the crown buds and crown branches. Later seedling inoculation tests showed that 1 isolate, while non-pathogenic to plants 6 weeks of age, was extremely pathogenic to seedlings. The other isolates pathogenic on seedlings were also pathogenic to older plants.

This information does not minimize the importance of *Rhizoctonia solani* as a pathogen on alfalfa but does serve to show that association of a *Rhizoctonia* isolate with a disease does not necessarily imply that it is the pathogen.

Effect of Rhizoctonia solani on alfalfa crowns.—In a field which had never been previously planted to alfalfa, California Common, Africa, Hilmar, and Briggs Common varieties of alfalfa were planted in rows 4–6 in. apart in plots 10 ft. wide by 12 ft. long. Both check and test plots were replicated. In May when the plants were 2 months old, 21 gm. of dried oat inoculum of a pathogenic *Rhizoctonia* isolate (ground in a Wiley mill with a 2mm. screen) was distributed in a shallow furrow close to each row of plants, except in the check plots. In July samples consisting of 150–200 plants, collected diagonally across each plot, were examined and each plant rated as to severity of root canker, stem canker, and crown bud necrosis. No measurable differences between varieties were detected and nearly all plants showed severe (over 50 per cent of the buds necrotic) crown bud necrosis and stem cankering. Leaves of plants with stem cankers often were yellow and sometimes slightly purple. Many cankered stems were girdled and soon died. Root cankers were not severe and were found only on occasional plants.

Discoloration of the woody tissue from 3–4 cm. above and below the lesion was observed frequently (Fig. 3A), and mycelium of *Rhizoctonia solani* was readily revealed in the vessels of the xylem tissue (Fig. 3B). Stems, each with a canker at the base were surface sterilized with $HgCl_2$ 1:1000, cut into 1 cm. sections with a sterilized knife, and placed on plates of acid PDA. In 2 experiments the fungus was isolated from 100 per cent of the cankered sections and from 38 per cent of the sections taken 3 cm. above the cankered sections. This showed that *Rhizoctonia* was able to migrate at least 3 cm. above the lesion. Longisections of the stems revealed the fungus in the xylem elements above and below the canker (Fig. 3B).

Discussion.—In California the most prevalent and important crown rot disease of the 3 described is dark crown necrosis for which a single causal agent has not been found. This suggests that the cause may be complex or due to physiological or mechanical factors. Numerous pathogenicity tests showed that *Fusarium roseum* or *F. solani* were not pathogens on alfalfa in California.

Stagonospora is potentially an important crown and root rotting fungus. Stagonospora crown and root rot, although potentially a lethal disease, should not be considered to be as important on alfalfa as the dark crown necrosis or Rhizoctonia stem canker. The sensitivity of the infection process as demonstrated by the low percentage of disease incidence when inoculum was placed in the soil may account for its sporadic nature. It is a root inhabitant and probably easily suppressed in the soil. Inoculation of roots with spores prior to incubation in a moist chamber for 2 days before transplanting to soil induced a high percentage of infections. Perhaps infection took place in 2 days, before the fungus was required to compete with the soil microflora. If a high amount of inoculum were present in a field and if conditions were favorable for infection, Stagonospora crown rot could be a serious disease.

Rhizoctonia, an omnipresent fungus in the soil, repeatedly infects crown buds, stems, and some isolates cause cankers on roots (25, 26). Diseases incited by the fungus may, if severe, be lethal to the plant; however, in most cases the effect is local. There is a possibility that the fungus, in killing tissue in advance of the hyphae, may incite a disease similar to the dark crown necrosis.

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LITERATURE CITED

1. Briton-Jones, H. R. 1925. Mycological work in Egypt during the period 1920-1922. Min. of Agr. Egypt, Tech. and Sci. Service, Bul. 49 (Bot. Sect.). (Rev. Appl. Mycol. **5**: 19-20. 1926).
2. Broadfoot, W. C. and M. W. Cormack. 1941. A low temperature basidiomycete causing early spring killing of grasses and legumes in Alberta. Phytopathology **31**: 1058-1059.
3. Brown, J. G. and F. Gibson. 1922. Plant pathology. 33rd Ann. Rept. Arizona Agr. Exp. Sta. for year ending June 30, 1922.
4. Cherewick, W. J. 1948. Two important diseases of alfalfa in Manitoba (Abs.) Phytopathology **38**: 5.
5. Cormack, M. W. 1937. Cylindrocarpon Ehrenbergi Wr. and other spp. as root parasites of alfalfa and sweet clover in Alberta. Can. Jour. Res. (C). **15**: 403-424.
6. Cormack, M. W. 1937. Fusarium spp. as root parasites of alfalfa and sweet clover in Alberta. Can. Jour. Res. (C) **15**: 493-510.
7. Cormack, M. W. 1945. Studies on Ascochyta imperfecta, a seed- and soil-borne parasite of alfalfa. Phytopathology **35**: 838-855.
8. Erwin, D. C. 1952. Pathogenicity of fungi associated with crown rot of alfalfa (Abs.). Phytopathology **42**: 513-514.
9. Fergus, E. N. and W. D. Valleau. 1926. A study of clover failure in Kentucky. Ky. Agr. Exp. Sta. Bul. 269: 142-210.
10. Garrett, S. D. 1950. Ecology of the root inhabiting fungi. Biol. Rev. **25**: 220-254.
11. Hanson, C. H. 1950. North Carolina, Summary of 1950 Report—Raleigh. In: 1950 report of the uniform alfalfa nurseries. U. S. Dept. Agr. (Comp. O. S. Aamodt) (processed).
12. Hanson, C. H. and J. L. Allison. 1951. Studies on

the nature and occurrence of stand depletion in alfalfa strains in North Carolina. Agron. Jour. **43**: 375-379.
13. Harris, M. R. 1941. Rosellinia root rot of alfalfa in California. U. S. Dept. Agr. Pl. Dis. Reptr. **25**: 407.
14. Hawn, E. J. and M. W. Cormack. 1952. Crown bud rot of alfalfa. Phytopathology **42**: 510-511.
15. Horsfall, J. G. 1930. A study of meadow crop diseases in New York. Cornell Agr. Exp. Sta. Mem. 130.
16. Johnson, E. M. and W. D. Valleau. 1933. Blackstem of alfalfa, red clover, and sweet clover. Ky. Agr. Exp. Sta. Bul. 339: 55-82.
17. Jones, F. R. 1950. Susceptibility of alfalfa plants to Collectotrichum trifolii. U. S. Dept. Agr. Pl. Dis. Reptr. **34**: 344.
18. Jones, F. R. and J. L. Weimer. 1938. Stagonospora leaf spot and root rot of forage legumes. Jour. Agr. Res. [U.S.] **57**: 791-812.
19. Kernkamp, M. F., D. J. de Zeeuw, S. M. Chen, B. C. Ortega, C. T. Tsiang and A. M. Khan. 1952. Investigations on physiologic specialization and parasitism of Rhizoctonia solani. Minn. Agr. Exp. Sta. Tech. Bul. 200.
20. Melchers, L. E. 1929. Alfalfa diseases in Kansas. Twenty-sixth Bienn. Report State Board of Agr. Kan. 31: 153-173.
21. Peterson, M. L. and L. E. Melchers. 1942. Studies on black stem of alfalfa caused by Ascochyta imperfecta. Phytopathology **32**: 590-597.
22. Remsberg, Ruth and W. W. Hungerford. 1936. Black stem of alfalfa in Idaho. Phytopathology **26**: 1015-1020.
23. Sanford, G. B. 1933. A root rot of sweet clover and related crops caused by Plenodomus meliloti D. and S. Can. Jour. Res. (C) **8**: 337-348.
24. Sleeth, B. 1951. Diplodia crown rot of alfalfa. U. S. Dept. Agr. Pl. Dis. Reptr. **35**: 50-51.
25. Smith, O. F. 1943. Rhizoctonia root canker of alfalfa (Medicago sativa). Phytopathology **33**: 1081-1085.
26. Smith, O. F. 1945. Parasitism of Rhizoctonia solani from alfalfa. Phytopathology **35**: 832-837.
27. Staten, G. and R. J. Leyendecker. 1949. A root rot disease of alfalfa caused by Fusarium solani. U. S. Dept. Agr. Pl. Dis. Reptr. **33**: 254-255.
28. Weimer, J. L. 1927. Observations of some alfalfa root troubles. U. S. Dept. Agr. Dept. Circ. 425.
29. Weimer, J. L. 1928. A wilt disease of alfalfa caused by Fusarium oxysporum var. medicaginis n. var. Jour. Agr. Res. [U.S.] **37**: 419-433.

A PHYTOPATHOLOGICAL NOTE

Use of Actidione in Culture of Plant Pathogenic Bacteria.[1] W. F. Jeffers. Use of actidione[2] (cycloheximide) for suppression of growth of fungi in isolating and maintaining cultures on non-phytopathogenic bacteria has been described.[3,4,5] A similar problem of fungus contamination occurs in the isolation and culture of plant pathogenic bacteria. Therefore, it was desirable to study the response of various bacterial plant pathogens and several fungi, commonly occurring as contaminants, to various concentrations of actidione in a culture medium.

A stock solution containing 0.01 gm./ml. was prepared by dissolving 0.5 gm. of actidione in 2.5 ml. of methyl alcohol in a sterile flask and adding 47.5 ml. of sterile, distilled water. Known amounts of nutrient-dextrose agar were prepared and when cooled to about 40° C, the stock solution was added to give concentrations of 0.1, 1.0, 10.0, 100.0, and 1000.0 μg./ml. (p.p.m.). (Addition of actidione in this manner is necessary since its activity is greatly decreased by autoclaving). The pH values of these media ranged from 5.8 to 6.2. Petri plates, containing about 10 ml. of medium were then prepared and inoculated with the desired organism. After 4 days at room temperature, growth in media containing actidione was visually evaluated by comparison with growth in medium containing no actidione.

Since growth of *Saccharomyces pastorianus*, a standard test organism for effectiveness of actidione, was reduced by 0.1 μg./ml. and almost eliminated at 1.0 μg., it was evident that the antibiotic was present and active. *Agrobacterium tumefaciens*, *Erwinia carotovora*, *Pseudomonas solanacearum* and *Xanthomonas campestris* made good growth at all concentrations tested. *Neurospora sitophila*, *Penicillium italicum*, *Rhizopus nigricans* and *Trichoderma viride* made no growth at a concentration of 100.0 μg. of actidione per ml. *Aspergillus niger* appeared to be the most tolerant to the antibiotic of the organisms tested. After 9 days the effect of actidione on these fungi was still evident but several had made slight growth at the 100.0 μg. concentration and *A. niger* had made slight growth in the 1000.0 μg./ml. concentration.

Use of actidione in culture media might prove to be a valuable practice in culture of plant pathogenic bacteria. Isolation of these organisms should be greatly facilitated and subsequent maintenance of uncontaminated cultures should be simplified. Another use of this material was found in the detection of contaminating bacteria in fungus cultures. Sometimes bacterial contamination, not readily visible was easily detected when such cultures were grown on media containing the higher concentrations of actidione. Here the bacteria grew readily while fungus growth was greatly reduced or eliminated.—Department of Botany, University of Maryland. College Park, Maryland.

[1] Scientific publication No. A437, contribution No. 2487 of the Maryland Agricultural Experiment Station, Department of Botany.

[2] A highly purified product furnished by the Upjohn Company, Kalamazoo, Michigan.

[3] Fuentes, C. A., F. Trespalacios, G. F. Baquero, and R. Aboulafia. 1952. Effect of actidione on mold contaminants and on human pathogens. Mycologia 44: 170-175.

[4] Georg, Lucille K., L. Ajello, and M. A. Gordon. 1951. A selective medium for the isolation of *Coccidioides immitis*. Science 114: 387-389.

[5] Phillips, G. B., and E. Hanel, Jr. 1950. Control of mold contaminants on solid media by the use of actidione. Jour. Bact. 60: 104-105.

SOME FACTORS AFFECTING DEVELOPMENT AND CONTROL OF ADAXIAL CRACK STEM OF CELERY [1]

J. B. Kendrick, Jr., R. T. Wedding, John T. Middleton, and Bernarr J. Hall

SUMMARY

Borax-containing sprays, when applied to celery at the proper growth stage, were shown to prevent development of adaxial crack stem of celery. Magnesium sulfate and manganese dioxide, applied as foliar sprays, were found to be ineffectual in preventing the disease and are not considered as likely factors in production of the disorder.

Time of application of borax sprays is important for prevention of adaxial crack stem. When plants were at the 5-6 leaf stage, spraying with 8.0 lbs. of borax per acre was ineffective in preventing the disorder whereas when plants were at the 10-12 leaf stage, spraying with borax at the same rate not only was effective in preventing the disease but also resulted in a significant increase in boron accumulation in leaf petioles.

Application of very high amounts of nitrogenous fertilizer (900 lbs. per acre) induced the occurrence of adaxial crack stem, whereas application of large amounts of potassium fertilizer (600 lbs. per acre) did not.

Neither application of borax to the soil nor reliance upon the presence of boron in irrigation waters is a practical means of correcting boron deficiency in celery because much of the boron is rendered insoluble by certain soils.

Adaxial crack stem of celery may be controlled by using moderate amounts of nitrogenous fertilizers and spraying plants in the 10-12 leaf stage or later with 8 lb. of borax per acre.

Borax sprays have been shown to arrest the development of adaxial crack stem of celery, *Apium graveolens L.*, (8, 9). The purpose of this paper is to report effects of nitrogen and potassium fertilizers upon incidence of and the relation of plant size and time of treatment to the development of adaxial crack stem as well as to report further upon the role of manganese and magnesium in this disorder.

MATERIALS AND METHODS.—The effect of abnormally high amounts of nitrogen and potassium fertilizers applied as side dressings at regular intervals during the growing season upon the incidence of adaxial crack stem was tested on Utah 10B celery, transplanted in August 1952 and maturing in February 1953. The plot, located near Oceanside in San Diego Co., consisted of 3 1-acre blocks all receiving an application of 35 cubic yards per acre of chicken manure prior to transplanting. Block A received a total of 900 lbs. of nitrogen, block B, 400 lbs. of nitrogen plus 600 lbs. of potassium, and block C, 400 lbs. of nitrogen. Three-hundred mature plants from 6 random areas in each block were examined for the occurrence of adaxial crack stem. The data were treated for statistical significance by the "t"-test.

The efficacy of micronutrient sprays containing borax, magnesium, or manganese applied to Utah 10B celery in the 5-6 leaf stage of growth for the prevention of adaxial crack stem was tested in a randomized and replicated field plot located near Carlsbad in northern San Diego Co. Manganese and magnesium were included to clarify their possible role in the control of adaxial crack stem. Inconclusive results were obtained earlier by Middleton et al (9, 10) in experiments where these materials had appeared to give occasional control of the disorder. The celery was transplanted in September 1952 and harvested in February 1953. The borax spray, containing 2 oz. of borax per gal. of water, was applied at a rate of 8.0 lbs. of borax per acre. Sprays containing ⅓ oz. of magnesium sulfate or ⅓ oz. of manganese dioxide per gal. of water were applied to celery independently at a rate of 1.4 lbs. of the chemical per acre. Untreated control plants were also included in the plot. The relation of plant size at the time of spray application to the development of adaxial crack stem was tested in an adjacent plot with similar design, planting and harvesting dates and treatments. Some plants were sprayed when they had 5-6 fully expanded leaves, some with 10-12 fully expanded leaves, and some at both growth stages. In both plots 50 mature plants in each replicated treatment were examined for the occurrence of the disease. Boron content of petioles was determined in the plant-size plot by the quinalizarin method described by MacDougall and Briggs (7) for each of 10 random plant samples taken from individual boron-treated and untreated plots. All data secured from these plots were analyzed by analysis of variance.

RESULTS.—The incidence of adaxial crack stem in the fertility plot in the block receiving 900 lbs. of nitrogen per acre, was significantly greater than that in the block receiving 400 lbs. of nitrogen per acre:

TABLE 1.—*Occurrence of adaxial crack stem of celery following soil treatments with abnormally high amounts of nitrogen and potash*

Block	Nitrogen lbs./A	Potash lbs./A	Disease Incidence %
A	900	0	8.0[a]
B	400	600	2.7
C	400	0	0.0

[a] Significantly higher than Block C at 19:1 by "t" test.

There were no significant differences in incidence of crack stem in 1 of the Carlsbad plots between the untreated control (7.2%), borax (4.7%), manganese

[1] Accepted for publication November 12, 1953.

TABLE 2.—*Effect of plant size at time of application of micronutrient sprays upon the boron content of mature Utah 10B celery and the incidence of adaxial crack stem*

Treatment	Concentration in: oz./gal. water	lbs./A.	Number of leaves at treatment	Disease Incidence %	Boron content in p.p.m. in petioles at maturity
Borax	2	8.0	5-6	6.8	25.5
Borax	2	8.0	10-12	0.0*	40.4*
Borax	2	8.0	5-6 and 10-12	0.0*	37.8*
MnO_2	⅓	1.4	5-6	2.8	----
MnO_2	⅓	1.4	10-12	3.2	----
MnO_2	⅓	1.4	5-6 and 10-12	2.8	----
$MgSO_4$	⅓	1.4	5-6	2.4	----
$MgSO_4$	⅓	1.4	10-12	2.4	----
$MgSO_4$	⅓	1.4	5-6 and 10-12	12.8	----
Untreated	—	—	————	8.8	22.5
Least significant difference at 19:1				7.2	12.8

*Difference from untreated significant at the 5 per cent level.

dioxide (6.3%) and magnesium sulfate (8.7%) when micronutrient sprays were applied at the 5-6 leaf stage.

There was a significant reduction in the amount of adaxial crack stem in the second Carlsbad plot in plants sprayed with borax in the 10-12 leaf stage (Table 2) but not in those receiving just 1 borax spray at the 5-6 leaf stage. There was no significant reduction in the incidence of disease in plants sprayed with manganese dioxide or magnesium sulfate.

The amount of boron accumulated in the tissues of plants receiving borax sprays at the 5-6 leaf stage was not significantly different from that in unsprayed control plants. However, there was a significant increase in the amount of boron in the tissues of celery plants that had been sprayed with borax at the 10-12 leaf stage only and plants sprayed at both the 5-6 and 10-12 leaf stage .

DISCUSSION.—It has been shown by Middleton et al (8, 9) and corroborated by Yamaguchi et al (13) that the development of adaxial crack stem can be arrested by spray applications containing boron. Evidence suggesting that boron plays a role in the development of this disease has been presented by Bardin (1, 2), Bardin and McCormick (3), Spurr (12) and Knott (5), although none of their work reports consistent field control of the disease. LaChance, Bertrand, and Perrault (6) report control of this disease in the acid muck soils of Canada with a soil application of 15 lbs. of borax per acre. However, applying borax to non-acid soils, such as those found in many celery growing regions of California, has often failed to prevent this disease.

Data secured from the Carlsbad plot where plants of different ages were sprayed with the 3 micronutrient sprays confirmed the earlier work of Middleton et al (9, 10) regarding the complete control of adaxial crack stem by boron-containing sprays and showed that manganese and magnesium were not effective in controlling its occurrence. In addition, this plot indicated the importance of plant size at the time of treatment in relation to subsequent development of the disease.

The fact that plants treated with borax at the 5-6 leaf stage contained no more boron in the petiole tissues than the untreated control plants indicates that at this stage of growth the plant is unable to accumulate sufficient boron to prevent a deficiency of this element as it approaches maturity. Plants with 10-12 fully expanded leaves at the time of treatment not only showed a significant elevation in petiole-boron content compared with untreated controls but also failed to develop the disease. The boron content of plants receiving sprays at both the 5-6 and 10-12 leaf stage was not significantly different from that of those receiving a single spray at the 10-12 stage. This is additional evidence that the early spray did not add to the normal accumulation of boron.

Adaxial crack stem rarely develops in young celery plants although this may occur where the availability of boron in the soil is extremely limited. Under these conditions plants should be sprayed with a borax-containing spray in order to prevent further development of the disease. This single application would probably not be sufficient to prevent the disease from developing as the plants approached maturity, so a second spray application at the 10-12 leaf growth stage would be necessary for complete control. A single application of 8.0 lbs. of borax per acre applied to plants with at least 10-12 fully expanded leaves will usually prevent development of this disease. It is quite possible that when borax sprays have not prevented the occurrence of adaxial crack stem as reported by Minges et al (11), the sprays were applied when the plants were too small and they were not followed with later applications, or else the dosage was inadequate.

Failure of borax to prevent the development of adaxial crack stem when applied to the soil, plus the development of crack stem despite the fact that irrigation waters used in celery culture usually contain sufficient amounts of boron to prevent any expression

of boron deficiency, is easily accounted for by the work of Eaton (4). He has shown that much of the boron present in soils is not readily soluble. He also has shown that soils are capable, in varying degrees, of removing from solution some of the boron applied by irrigation waters. He states that the nature and quantity of soluble or slightly soluble boron compounds in the soil determine soil-solution concentrations, and it is the latter concentrations that affect plant growth. It would appear unlikely, therefore, that one could predict the success of soil application of borax for the correction of adaxial crack stem without having a comprehensive knowledge of the soil in question and its capacity to furnish available boron in the soil solution. In view of this fact, application of boron solutions directly to the growing plant by foliar sprays is the most practical method of providing the plant with sufficient boron to permit normal growth.

The significantly increased incidence in celery adaxial crack stem in the field plot where an abnormally high amount of nitrogen fertilizer was used confirmed the observations of Bardin (2) and Middleton et al (9, 10) that the disease seemed to be associated with the use of very large amounts of nitrogenous fertilizers. Yamaguchi et al (13) also confirmed these observations.

The use of extremely high quantities of nitrogen accompanied by optimum growing conditions often accelerates plant growth rate and results in an earlier maturity than normal. It is probable that the boron requirements of the plants sometimes exceed the available supply in the soil solution under these conditions. The total supply of boron in the soil is usually sufficient for normal growth but is not solubilized rapidly enough to satisfy the requirements of an accelerated plant growth rate, and a boron-deficient condition develops.

It seems likely that potassium *per se* is not a major factor in favoring the development of adaxial crack stem because there was no significant increase in the incidence of the disease where very high amounts of potassium fertilizer were used. Excessive amounts of potash were reported by Middleton et al (8) as causing no increase or decrease in the amount of the disease. It was reported by Yamaguchi et al (13) that excessive potassium soil application may be a factor only when accompanied by large amounts of nitrogenous fertilizers. If the potassium supply in some soils is a limiting factor in plant growth rate and potash additions contribute to an accelerated growth rate the disease may be induced in the same manner as with nitrogen additions.

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LITERATURE CITED

1. BARDIN, R. 1947. Association of split heart or doubles with crack-stem of celery. U. S. Dept. Agr. Pl. Dis. Reptr. **31**: 119-120.
2. BARDIN, R. 1948. Crack-stem of celery in Salinas Valley. U. S. Dept. Agr. Pl. Dis. Reptr. **32**: 15-16.
3. BARDIN, R., AND J. A. MCCORMICK. 1949. Boron nutrition of celery. Western Grower and Shipper **20(5)**: 20-21.
4. EATON, F. M. 1935. Boron in soils and irrigation waters and its effect on plants. U. S. Dept. Agr. Tech. Bul. 448.
5. KNOTT, J. E. 1953. The problem of "brown checking" of celery. Hort. Abs. **23**: 109.
6. LACHANCE, R. O., P. BERTRAND, AND C. PERRAULT. 1942. Manifestation extrême de la qerçure des pétioles du céleri. Sci. Agr. **23**: 187-193.
7. MACDOUGALL, G., AND D. A. BRIGGS. 1952. Estimation of boron in plant tissue. Modification of the quinalizarin method. Anal. Chem. **24**: 566-569.
8. MIDDLETON, J. T., B. J. HALL, R. T. WEDDING, AND J. B. KENDRICK, JR. 1952. Celery brown checking curb seen. West. Grow. and Ship. **23(10)**: 42-44.
9. MIDDLETON, J. T., B. J. HALL, R. T. WEDDING, AND J. B. KENDRICK, JR. 1952. Adaxial crack stem of celery. Phytopathology **42**: 603-606.
10. MIDDLETON, J. T., J. B. KENDRICK, JR. AND B. J. HALL. 1952. Parasitic and nonparasitic diseases of celery in southern California. U. S. Dept. Agr. Pl. Dis. Reptr. **36**: 188-193.
11. MINGES, P. A., M. YAMAGUCHI, J. E. KNOTT, A. R. SPURR, AND J. E. WELCH. 1952. Brown checking of celery. Calif. Agr. **6**(12): 5-6.
12. SPURR, A. R. 1952. Fluorescence in ultraviolet light in the study of boron deficiency in celery. Sci. **116**: 421-423.
13. YAMAGUCHI, M., F. W. ZINK, AND A. R. SPURR. 1953. Cracked stem of celery. Calif. Agr. **7**(5): 12.

PLUGGING OF VESSELS BY TYLOSES AND GUMS IN WILTING OAKS [1]

B. Esther Struckmeyer, C. H. Beckman, J. E. Kuntz, and A. J. Riker

SUMMARY

Certain fundamental changes within wilt-infected northern pin oaks were studied by histological methods during various stages of disease development.

Extensive plugging of the xylem vessels with tyloses and gums preceded foliage wilt. Tyloses formed in the large vessels of the spring wood, especially of the last annual ring, but less so in the small vessels of the summer wood. In the later stages of wilt, gum often formed in the smaller vessels, vascular rays, xylem parenchyma, tracheids, and among the tyloses in the larger vessels. Vascular plugging of the roots appeared less extensive.

The development of foliage wilt followed extensive vascular plugging and appeared to result from insufficient water rather than from a toxic substance.

Sudden wilting and death of infected trees are characteristic of the oak wilt disease, especially in species of the red oak group. In northern pin oak, symptoms may appear within 2 or 3 weeks following inoculation with the inciting fungus *Chalara quercina* Henry (*Endoconidiophora fagacearum* Bretz) (11). The entire crown soon is affected. Premature defoliation and death of such trees follow rapidly.

Symptom expression in northern pin oak may vary considerably during the course of the season. In the spring, the first symptoms appear on leaves in the upper and outer portions of the crown of infected trees. These leaves become flaccid during the day and recover at night for a period of 3 or 4 days. These initial symptoms are followed by a rapid collapse, shrivelling, and desiccation of the leaves. Little or no marginal bronzing of the leaves occurs at this time of year.

During the summer, after leaves have matured, diurnal drooping and recovery of leaves appear also for a period of 3 to 4 days. Later, conspicuous, progressive, marginal bronzing develops. The leaves die, but do not collapse.

In late summer and early fall, premature yellowing, death and defoliation occur. When this coloration coincides with normal fall coloration, only the extensive and premature defoliation distinguishes the infected tree from a healthy tree.

Certain host responses to infection by the oak wilt fungus have been described by Beckman et al. (3). Upward movement of radioactive isotopes in the sap streams of inoculated trees was reduced by 85 per cent 3 days before permanent wilt appeared. A comparable reduction was found in the flow of water through twig sections of infected trees. The evidence indicated that a disturbance within the vascular system of infected oaks might account for the rapidity and severity of symptom development.

In other vascular wilts, plugging of the xylem vessels frequently has been associated with symptom development. The formation of tyloses and gums has been reported in pathological wilts of many trees. Such vascular plugging has been observed in verticillium wilt of elm and maple (13), persimmon wilt (7), mimosa wilt (10), the Dutch elm disease (1, 2, 4, 6, 12, 14), and others. A thorough investigation of anatomical changes in phony peach disease and in Pierce's disease of grape has been reported by Esau (8). The changes associated with these diseases were deposition of gum in the xylem cells and occlusion of the xylem vessels with tyloses.

Tyloses occur normally in many plants. Gerry (9) described tylose formation in many native North American trees, including oak. In the white oaks, tyloses were generally abundant, even in the outermost rings. Tyloses occurred commonly in the sapwood as well as in the heartwood of many trees. In some instances, tyloses were stimulated by wounding or other injuries. A survey of tylose and gum formation in 1100 plant genera, as well as a critical study and review of normal tylose formation, has been presented recently by Chattaway (5). It was concluded that both tyloses and gums were formed by ray parenchyma cells, and that the size of the pit aperture determined whether tyloses or gum were formed. Blocking of the vessels by gum and tyloses appeared to be a manifestation of the reaction of living ray cells to a stimulus which caused increased activity.

The purpose of this study was to determine, by histological methods, any fundamental changes within the host which were associated with infection by the oak wilt organism.

MATERIALS AND METHODS.—Materials for this investigation were obtained from 20-year-old northern pin oaks (*Quercus ellipsoidales* Hill) in experimental woodland plots in central Wisconsin. These trees were inoculated at various intervals prior to sampling by introducing heavy conidial suspensions into the xylem of the root collar region. Branch and root sections ¼ to 1 in. in diameter were obtained from healthy trees and from inoculated trees which showed various stages of wilt. Leaves from these branches were collected also. Samples were fixed in formal-acet-alcohol or were placed in water for immediate examination. The fixed samples were dehydrated with n-butyl alcohol

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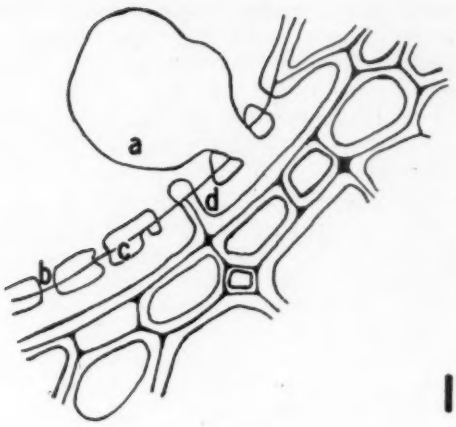

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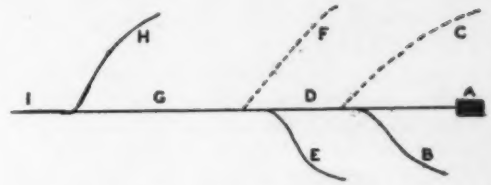

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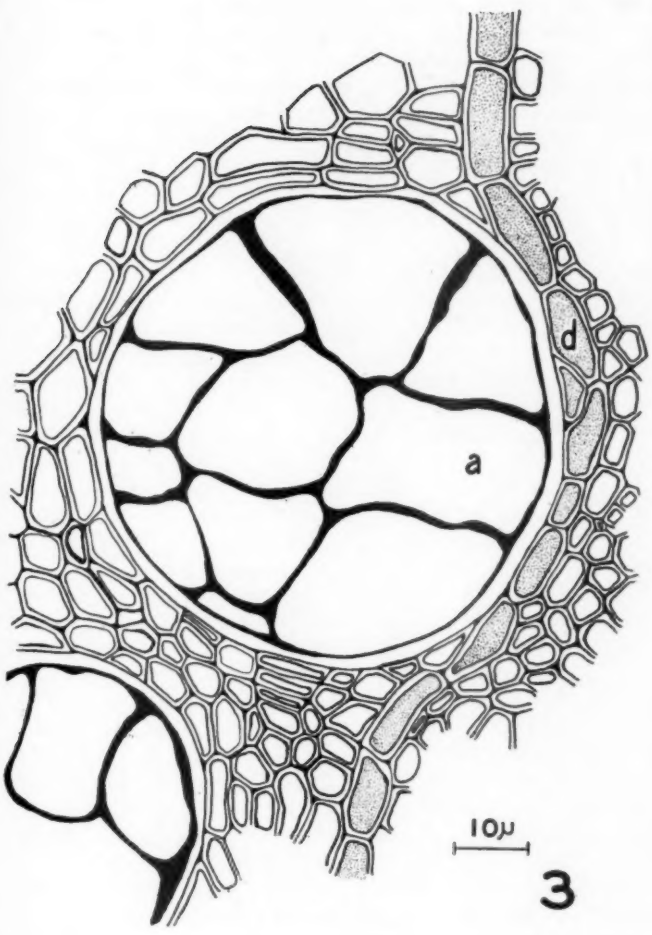

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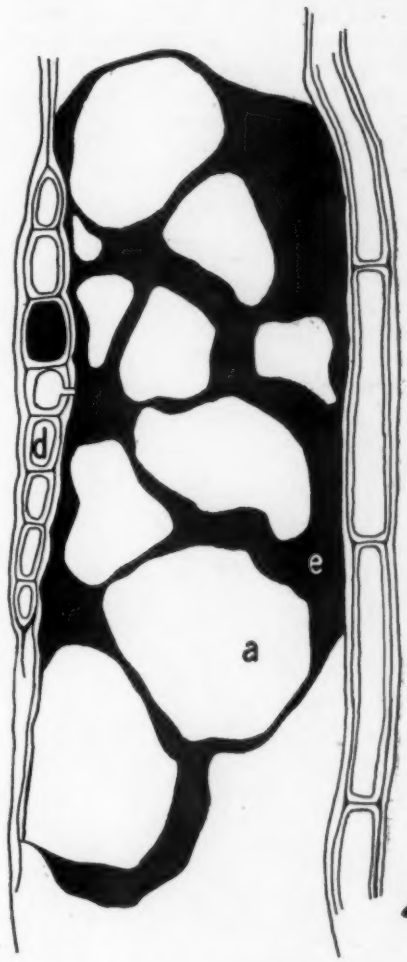

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FIGS. 1-4. (1, 3, and 4 are camera lucida drawings magnified 960 ×. 1 and 3 are transverse sections of stems from northern pin oak; 2 is a sketch of branch with 5 laterals inoculated at tip of main branch; 4 is a longitudinal section of a xylem vessel.) Fig. 1. Portion of a vessel showing a tylose formed by the expansion of the pit-closing membrane between the vessel wall and the ray cells. a. tylose; b. pit-closing membrane; c. secondary wall; d. ray cell.—Fig. 2. Branch with 5 laterals. Two of the laterals (C, F) showed wilt symptoms, and a considerable number of tyloses; B, E, and H showed no evidence of wilting, although tyloses were apparent in the vessels. Fig. 3. Vessel blocked with tyloses. Tylose walls become lignified and frequently pitted. Ray cells adjacent to the vessel: a. tylose; d. ray cell.—Fig. 4. Portion of vessel with both tyloses and gum deposits contributing to the occlusion of the vessel. a. tylose; e. gum deposit.

and infiltrated with paraffin. Sections 20μ in thickness were cut on a sliding microtome and stained with safranin and fast green for detailed studies of the wood. Gum was identified with phloroglucinol and hydrochloric acid. Iodine-potassium iodide was used to test for starch. Fresh samples were cut on a sliding microtome, stained with an aqueous solution of safranin, and mounted in glycerine. Both tyloses and gum acquired a red color with this stain so that the position and relative amounts of each were detected readily.

OBSERVATIONS.—*Anatomy of branches.*—The wood anatomy was studied in several samples from each of 10 healthy check trees, 16 with early wilt, and 17 with severe wilt symptoms. The wood of healthy northern pin oak was of the ring porous type with conspicuous annual rings. It consisted of vessels with bordered pits, libriform fibers with very thick walls and reduced pits, thinner walled tracheids with numerous bordered pits, and 3 or more layers of xylem parenchyma surrounding the vessels. Vascular rays were commonly adjacent to or near the vessels. These were usually uniseriate and extended throughout the vascular tissue. Multiseriate rays were less numerous. As the annual growth progressed during late summer, smaller and fewer vessels were formed. Tyloses and gum were not observed in any of the normal stems examined.

Tyloses were formed in the vessels of inoculated trees which showed various degrees of wilt. These resulted from the extension into the vessel lumen of the pit-closing membrane of the pit pairs of ray parenchyma cells and the vessels (Fig. 1). In early stages of development, tyloses appeared as small outgrowths along the vessel wall. These outgrowths continued to expand into spherical and elongate structures within the lumen of the vessel (Fig. 10). The tylose walls eventually came into contact with each other and occluded the vessels completely. The tylose walls then became thickened and lignified (Fig. 3). In some instances normal pits were differentiated in the tylose walls. The nucleus of the ray cell often migrated into a tylose. Starch grains were present occasionally.

The characteristics and extent of tylose development in vessels were studied further from longitudinal sections. Specimens from 6 wilting trees were examined. In early stages of wilt, tyloses were small and formed along parts of the wall immediately adjacent to ray cells. Soon, however, the entire lumen was occluded (Fig. 7). Tyloses were formed in the large vessels of the spring wood but less so in the small vessels of the summer wood. Generally they were found only in the vessels of the last formed annual ring, except in severe cases of wilt when they were apparent also in the second annual ring.

Gummosis also was apparent in the diseased samples. This occurred in the later stages of wilt, especially in the small vessels of the summer wood. Gum was found also in vascular rays, xylem parenchyma, and tracheids. Vascular rays, particularly in the outer annual ring, frequently were occluded with this substance. Lesser amounts were commonly found in tracheids and xylem parenchyma. When occlusion was severe, tyloses and gum were often found together in the same vessels (Fig. 4).

Gum was first apparent in vessels as small droplets along the wall. Gradually these droplets enlarged and coalesced until they filled the cell completely. The gum was homogeneous and stained a reddish color with phloroglucinol and hydrochloric acid.

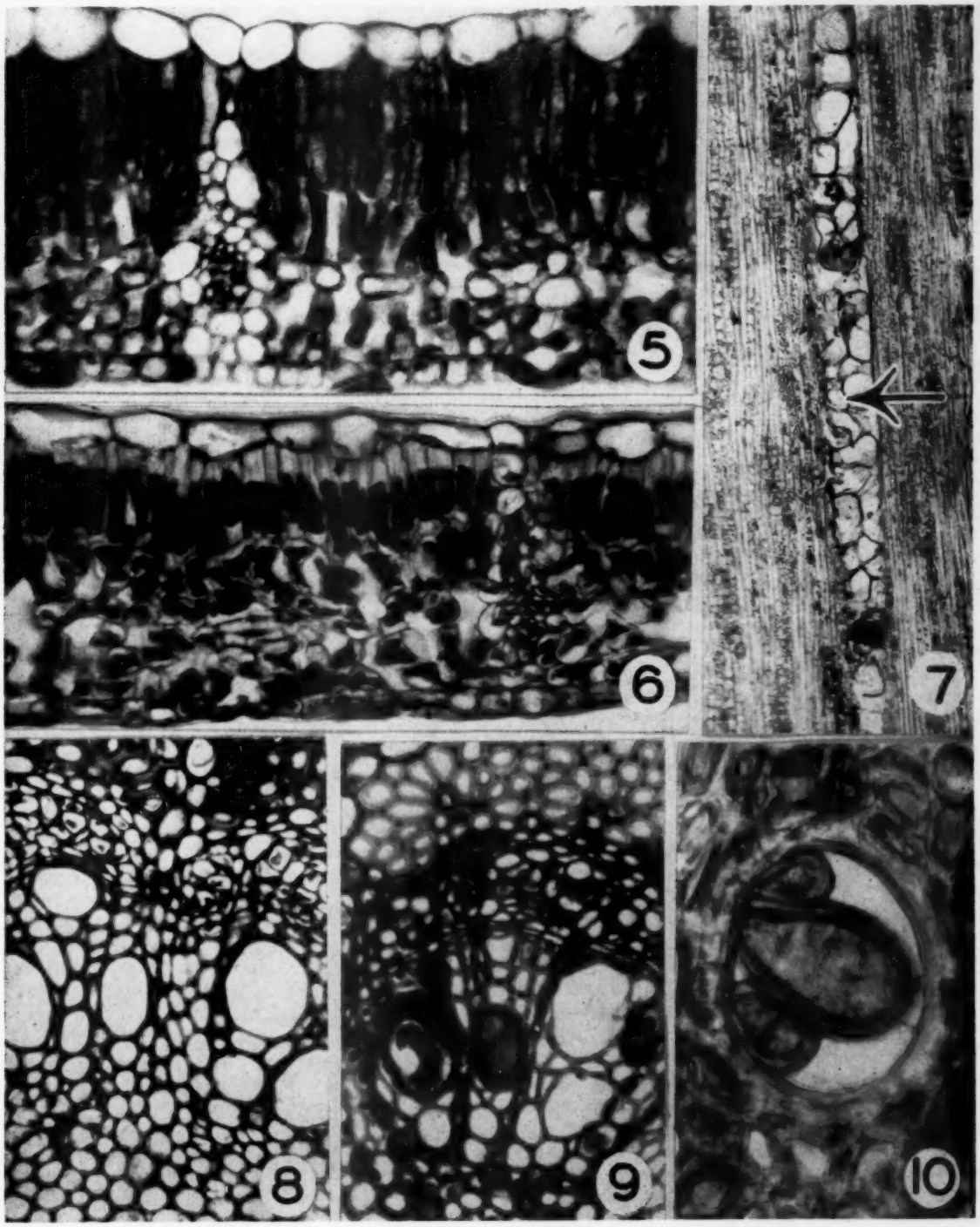
Anatomy of leaves.—Leaves from 3 healthy and 5 diseased oaks were examined. Marked disturbances in the cellular structure were evident in the petioles and leaves of trees showing wilt symptoms. Severe plasmolysis of the protoplast was characteristic in all cells of the wilting leaves. Wilting leaves (Fig. 6) were thinner than healthy ones (Fig. 5) as a result of plasmolysis and contraction of the walls of the palisade and spongy parenchyma cells. Plastids were easily distinguishable in healthy leaves, but could not easily be identified in infected ones. The vascular tissue of the leaf blade was shrunken, misshapen, but devoid of tyloses. Vessels in the petioles, however, were commonly occluded with tyloses and gum (Fig. 8, healthy; and Fig. 9, diseased). Ray cells adjacent to the xylem vessels of the petioles commonly were collapsed.

Anatomy of roots.—Vessels in 5 roots from each of 3 healthy trees and 5 inoculated trees which showed incipient wilt were without tyloses. The majority of root samples from 5 trees with severe wilt contained tyloses, although the number of vessels plugged was considerably less than in the stems. Multiseriate rays separated the xylem elements into several sectors. Frequently the vessels in some of the sectors were occluded with tyloses, while vessels in other sectors showed no evidence of tylose formation. In some roots all annual rings were affected. In others tyloses were present only in the more recently formed vessels. Gum was generally absent in the roots except in very severe stages of wilt. Roots contained considerable starch. A slight amount of this was present in the tyloses.

Tylose formation and symptom development.—The relationship between the time and extent of tylose-formation and symptom development was studied in individual, locally-inoculated branches. The inoculated branches were examined for evidences of vascular plugging while symptoms were still confined to their distal parts.

One 4–5 ft. branch on each of 20 northern pin oaks was inoculated as follows. The distal end was cut at a point where the branch was approximately ¼ in. in diameter. A test tube containing a heavy conidial suspension was slipped over this cut end and fastened in place. The branch was tied down until the suspension had been taken up. Uptake was rapid. Two to 3 weeks later, 2 of these branches which showed incipient wilt on several twigs and 4 which showed

no symptoms were examined for tylose formation. Each twig and each segment of the branch between these twigs was sampled. At the time the fungus was isolated readily from all secondary twigs.



FIGS. 5-10. (Photomicrographs of normal and diseased parts of northern pin oak. 5, 6, 8, 9, 10 transverse sections; 7 longitudinal section.) Fig. 5. Normal leaf. ×375.—Fig. 6. Leaf from severely wilted tree. Leaf thinner than normal as a result of plasmolysis of the protoplast and shrinking of the cells. Chloroplasts were not distinguishable. ×375.—Fig. 7 Wood of severely wilted oak showing xylem vessel completely occluded with tyloses (arrow). ×65.—Fig. 8. Petiole of normal leaf. ×375.—Fig. 9. Petiole of leaf from severely wilted tree. Tyloses in some of the vessels. Gum in some of pith cells, tracheids and phloem. ×375.—Fig. 10. Vessel with 3 tyloses occluding the lumen. Tyloses may start as spherical or elongate structures. Ray cells are adjacent to the vessels. ×670.

The severity of wilt in the lateral branches which showed symptoms correlated with the degree of tylose formation. Few tyloses appeared in the 4 branches which showed no symptoms. Abundant tyloses were present in branches which were wilting. In 1 representative branch (Fig. 2), secondary twigs B, E, and H appeared healthy, but contained some to many tyloses. Up to 50 per cent of the vessels were occluded. Twigs C and F, which were opposite these, showed incipient wilt. Fifty to 75 per cent of the vessels in any single cross section (20μ thick) from these branches were occluded. Tyloses were most severe in the outer annual ring and common in the second annual ring. Only in severe wilt were tyloses formed in the third annual ring. Frequently tyloses were formed only in a part of the circumference of the main branch. Lateral branches originating from this severely plugged sector of the main branch showed wilt symptoms.

Gum commonly was associated with tyloses in the larger vessels when occlusion was severe. Under these conditions, gummosis occurred also in the smaller, later formed vessels and in the xylem parenchyma.

Discussion.—Tylose and gum formation were consistently associated with wilt in northern pin oaks. Tyloses formed extensively in large vessels, but were less common in the smaller vessels. None were found in the tracheids. On the other hand, gummosis was found in tracheids and in the smaller vessels, but only occasionally in the larger vessels, and then when tyloses were abundant. These findings agree with the conclusions of Chattaway (5) that tyloses and gums have a common incitant and that the type of manifestation (tylosis or gummosis) depends upon pit size.

Considerable plugging occurred before wilt became apparent. Tyloses were abundant in the large vessels of the outer 1, and sometimes in the outer 2, annual rings. The vessels of these outer annual rings provide most of the water conduction necessary for the survival of the tree. As seen in cross section, not all the vessels in these outer annual rings were plugged, or, as seen in longitudinal sections, not all the length of the vessel lumen was always plugged. However, considering the vessel or sapwood as a whole, the additive effect of plugging in different parts appeared sufficient to reduce the sap flow in the outer wood to an extremely low level before leaf symptoms appeared.

Further evidence concerning the relationship between the time of tylose and gum formation and that of leaf symptom development was obtained from studies with localized branch inoculations. Those with severe tylose formation showed wilt symptoms, whereas those with slight to moderate tylose formation had normal appearing foliage. This indicated that considerable vascular plugging preceded symptom development.

The diurnal drooping and recovery which preceded permanent wilting are typical of disturbed water relationships of many types. The shrunken tissue and plasmolysis of cells in diseased leaves found in this study indicate also that a water shortage may be involved.

A severe water shortage in wilt-infected oaks has been reported (3). An 85 per cent drop in the movement of radioactive rubidium in infected trees was found during a period 3 and 4 days preceding first permanent leaf symptoms. A drop in water-flow through twig sections was comparable in both time of occurrence and extent. A further, gradual drop in the water-carrying capacity occurred during the development of initial permanent wilt and severe wilt.

The results obtained in this study indicate that this severe drop in water-carrying capacity of the xylem tissue which preceded leaf symptoms in infected oaks was caused by the formation of tyloses and gum. Plugging reduced the water supply to the wilting threshold. Diurnal drooping and recovery of leaves resulted. A continued development of tyloses and gum produced further plugging which brought about permanent wilt.

The nature of the fungus metabolites operating in this disease has not been defined. The progressive bronzing of leaves of wilting trees which occurred during the summer might suggest the accumulation of necrosis-causing "toxins." However, no evidence of such necrosis was apparent in leaf symptoms during the spring and fall. Furthermore, the tyloses (and the ray cells from which they developed) were well exposed to any fungus metabolites which were present in the vessels. Nevertheless, lignified walls and pits comparable to those in normal cells were differentiated in these tyloses. This has suggested that a modification of the normal host cell metabolism has taken place rather than that some drastic toxic action has occurred.

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LITERATURE CITED

1. Ahmet, H. 1933. Untersuchungen über Tracheomykosen. Phytopath. Ztschr. **6**: 49-101.
2. Banfield, W. M. 1941. Distribution by the sap stream of spores of three fungi that induce vascular wilt diseases of elm. Jour. Agr. Res. [U.S.] **62**: 637-681.
3. Beckman, C. H., J. E. Kuntz, A. J. Riker, and J. G. Berbee. 1953. Host responses associated with the development of oak wilt. Phytopathology **43**: 448-454.
4. Buisman, Christine. 1928. De oorzaak van de Ieperiziekte. Tijdschr. Nederl. Heidemaatsch **10**. 7 p.
5. Chattaway, M. Margaret. 1949. The development of tyloses and secretion of gum in heartwood formation. Austral. Jour. Sci. Res. (Series B) **2**: 227-240.
6. Clinton, G. P. and Florence A. McCormick. 1936. Dutch elm disease. Conn. Agr. Exp. Sta. Bul. 389: 701-752.
7. Crandall, B. S. and W. L. Baker. 1950. The wilt disease of American persimmon, caused by Cephalosporium diospyri. Phytopathology **40**: 307-325.
8. Esau, Katherine. 1948. Anatomic effects of the viruses of Pierce's disease and phony peach. Hilgardia **18**: 423-482.

9. GERRY, ELOISE. 1914. Tyloses: their occurrence and practical significance in some American woods. Jour. Agr. Res. [U.S.] **1**: 445-469.
10. HEPTING, G. H. 1939. A vascular wilt of the mimosa tree (Albizzia julibrissin). U. S. Dept. Agr. Cir. 535. 11 p.
11. KUNTZ, J. E., C. H. BECKMAN, AND A. J. RIKER. 1952. Oak wilt development in relation to time and place of inoculation and concentration of inoculum. (Abs.) Phytopathology **42**: 13.
12. POPE, S. A. 1944. Some studies on the Dutch elm disease and the causal organism. (Abs.) Theses of Cornell Univ. p. 347-348.
13. VAN DER MEER, J. H. H. 1926. Verticillium-wilt of maple and elm seedlings in Holland. Phytopathology **16**: 611-614.
14. ZENTMYER, G. A. 1942. Toxin formation by Ceratostomella ulmi. Science **95**: 512-513.

PRODUCTION OF VASCULAR BROWNING BY METABOLITES FROM SEVERAL PATHOGENS[1]

N. N. Winstead and J. C. Walker

SUMMARY

The Fusarium wilt organisms inciting tomato wilt, cotton wilt, cabbage yellows, pea wilt, and pea near wilt were grown in wheat bran cultures. The filtered washings were assayed for pectinmethylesterase and polygalacturonase, and each filtrate was introduced into cut stems of resistant and susceptible varieties of all 4 species of plants. All showed high pectinmethylesterase (PME) and low polygalacturonase (PG) activity, and each induced vascular browning in all test plants. Similar results were obtained with the fusaria inciting root rots of pea, bean, and brome grass.

Less enzyme activity of both enzymes was found in replacement culture filtrates for each of the previously mentioned organisms and the replacement filtrates also induced less browning in test plants than did the bran filtrates. No browning was secured when filtrates were heated for 10 minutes at temperatures above 60° C., and no PME activity was found in such filtrates.

The highest PME activity was found in *Pseudomonas solanacearum* potato-dextrose broth culture filtrates. The high production of PME and the relatively larger proportion of PG may account in part for the rapidity with which the wilt disease incited by this organism progresses in its host plants.

No demonstrable quantity of PME activity was noted in replacement culture filtrates of *Botrytis cinerea* and *Alternaria solani*, and these filtrates induced no browning. Bran culture filtrates of *B. cinerea* were high in both enzymes and induced some browning and soft rot of the stems of test plants. *A. solani* bran filtrates were relatively low in both enzymes.

The browning factor was common to all the wilt and root-rot organisms and was not specific for the respective susceptible host. The establishment of the pathogen in its respective susceptible host plant is therefore due to other factors than PME activity, but after establishment in the xylem the latter enzyme appears to be important in initiating vascular browning and plugging.

In a study of *Fusarium* wilt induced by *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder and Hansen on tomato (*Lycopersicon esculentum* Mill.), Scheffer and Walker (7) showed that vascular plugging and browning characteristic of the disease were induced by inserting cuttings of tomato stems in a filtrate from a 24-hour replacement culture of the pathogen. The metabolite concerned was destroyed by exposure to 60° C. for 10 minutes, and it was not removed by dialysis for 15 hours. Later work (3) indicated that the plugging was brought about by the action of pectic enzymes secreted by the fungus on the xylem cells. The browning appeared to result from the action of phenolic substances released from the walls which were polymerized to melanins by the action of polyphenoloxidases naturally present in the host cells. Gäumann et al (2) isolated a vascular browning entity which was named vasinfuscarin and suggested that it was an enzymatic protein. When cuttings of varieties of tomato resistant and susceptible to *F. oxysporum* f. *lycopersici* were tested with the filtrate they reacted similarly (7). This indicated that the resistant variety possessed no mechanism to cope with the disease-inducing fungus metabolite concerned if the fungus became established in the vascular system of the resistant plant and grew as effectively as in the susceptible plant. In other words the resistance of certain tomato varieties to wilt was not explained on the basis of tolerance to the pectic enzymes.

In the present investigation the study was extended to several other vascular pathogens, 3 root-rotting fungi, and 2 leaf-blighting fungi, in their effects upon several plant species. A preliminary report has been published (8).

METHODS AND MATERIALS.—Test plants were chosen from varieties of tomato, cotton, (*Gossypium hirsutum* L.), pea (*Pisum sativum* L.), and cabbage (*Brassica oleracea* var. *capitata* L.) which were resistant or susceptible to the vascular fusarial diseases of the respective host plants. These included Jefferson (resistant) and Bonny Best (susceptible) tomato; Coker 100 (resistant) and Stoneville (susceptible) cotton; Davis Perfection pea (susceptible to wilt and near-wilt);

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Wisconsin Perfection pea (resistant to wilt and susceptible to near-wilt), and New Era pea (resistant to wilt and to near-wilt); Jersey Queen (resistant) and Copenhagen Market (susceptible) cabbage. The plants were grown in the greenhouse in 4-in. clay pots in white silica sand. They were watered with 1H nutrient solution described elsewhere (4).

A highly pathogenic strain (R5-6) and a mildly pathogenic one (A15-8) of the tomato wilt organism were used. Other vascular pathogens included were the cabbage yellows organism (*Fusarium oxysporum* f. *conglutinans* (Wr.) Snyder and Hansen, race 1); the pea wilt fungus (*F. oxysporum* f. *pisi* (Linford) Snyder and Hansen, race 1); the pea near-wilt organism (*F. oxysporum* f. *pisi* (Linford) Snyder and Hansen, race 2); the cotton wilt fungus (*F. oxysporum* f. *vasinfectum* (Atk.) Snyder and Hansen); the bacterial wilt pathogen of tomato and many other hosts (*Pseudomonas solanacearum* E. F. Sm.). Additional organisms studied were: *Fusarium solani* f. *pisi* (F. R. Jones) Snyder and Hansen, which incites Fusarium root rot of pea; *F. solani* f. *phaseoli* (Burk.) Snyder and Hansen, which incites Fusarium root rot of bean; a strain of *F. oxysporum* Schlecht., which incites root rot of brome; *Alternaria solani* (Ell. and Mart.) L. R. Jones and Grout, which incites early blight of potato and tomato; an isolate of *Botrytis cinerea* Fr., which incites a foliage blight of potato in the greenhouse.

Cuttings were taken from tomato and cotton plants 12–20 in. tall and 6–8 weeks old. Cabbage and pea plants of various sizes with 2–12 expanded leaves were used. The plants in each test were carefully selected for uniformity. The stems were cut under water with a razor blade and the cut ends placed in the test solutions.

The fungi were grown in 32-oz. pharmaceutical bottles, placed on the side to give the greatest possible exposure to the air. Each bottle contained 100 ml. of liquid medium. Cultures were seeded with small pieces of mycelial mat and were grown without shaking at 28° C. for 2 weeks. Unless otherwise indicated, the standard culture medium, a modified Richard's solution, contained NH_4NO_3 (10 gm.); KH_2PO_4 (5 gm.); $MgSO_4 \cdot 7H_2O$ (2.5 gm.); $FeCl_3 \cdot 6H_2O$ (.02 gm.); cerelose (50 gm.); water (1000 ml.). The initial pH of the medium was pH 3.8–4.0.

After 2 weeks the culture fluids were filtered off in a Büchner funnel and discarded. The mycelial mat was washed in distilled water and suspended in 100 ml. of a 1:4 dilution of Czapek's solution. This diluted solution contained $NaNO_3$ (2 gm.); KH_2PO_4 (1 gm.); KCl (.5 gm.); $MgSO_4$ (.5 gm); $FeSO_4$ (.01 gm.); cerelose (20 gm.); water (5000 ml.). After 24 hours in this replacement medium the culture fluids were filtered through a layer of celite and asbestos to remove all fungus or bacterial cells.

Bran culture filtrates were obtained by growing the organisms on 25 gm. of sterile moist wheat bran in 32-oz. pharmaceutical bottles for 3-6 days. Water (200 ml.) was then added with 2 drops of toluene and allowed to autolyze for 12 hr. after which time the extract was filtered through cheesecloth and centrifuged. The clear supernatant was used.

Cuttings were usually placed in the filtrate at 27°–28° C. and approximately 50 per cent relative humidity for 2–4 days, but some tests were conducted in the greenhouse where temperature and humidity conditions varied. The cuttings were usually left in the filtrate for 2 days, then placed in water for 2 days. However, if the test plants showed no evidence of wilt they were left in the filtrate for 3 or 4 days. The bases of the stems were trimmed once each day. Changes of filtrate were made twice daily to reduce the growth of contaminants.

On the fourth day the cuttings were rated according to the intensity of vascular browning as follows: 0 — no vascular browning; 1 — slight browning at the base of the stem or in some leaf axils; 2 — browning in the lower third of the stem; 3 — browning in the lower ⅔ of the stem; 4 — browning from the base to the apex of the stem.

Four to 6-day-old bran and 24-hr. replacement culture filtrates were assayed for pectic enzymes. The following pectinmethylesterase (PME) assay method based on the hydrolytic removal of methyl groups from the pectin molecule by the action of the enzyme was used. Exactly 10 ml. of 0.5M acetate buffer (pH 4.5) were added to 75 ml. of 1.5 per cent pectin solution in a 250-ml. beaker. The entire filtrate or enzyme sample was adjusted to pH 7.0 prior to addition to the pectin-buffer mixture. The titre was recorded and a correction for the dilution was made in final calculations. Assay samples of 5, 10, and 15 ml. (brought up to 15 ml. in each case with distilled water to keep the volume constant) were added to the pectin buffer mixture with constant stirring. A check was run in which 5, 10, and 15 ml. of a heated sample were substituted. After being held at 31° C. for 3 hours, the solutions were titrated, using mechanical stirring, to pH 7.0 with 0.1N alkali. The difference between the number of ml. of 0.1 N alkali required for the sample and that required by the heated check equals the ml. of 0.1N acid released by enzyme activity. This amount multiplied by 3.1 gives the mg. methoxyl removed by the enzyme sample in 3 hours. A correction for the dilution factor and ml. of the sample was made which yielded the PME activity in mg. methoxyl removed per ml. of culture filtrate.

The polygalacturonase (PG) assay was a modification of the method of Jansen and MacDonnell (5). This assay was based on the increase in reducing value as a result of glycosidic hydrolysis of the pectin molecule and was carried out at 30° C. Five-ml. aliquots were removed at definite time intervals (0, 15, 30, 60 and 120 minutes, etc., depending on the activity of the filtrate) from a filtrate-sodium polypectate solution. This mixture contained 10 per cent filtrate and the final concentration of pectate was 0.5 per cent. Each aliquot was added to 1.8 ml. of 1M $NaCO_3$, which stopped the enzyme activity, in glass-stoppered flasks.

TABLE 1.—*Effect of replacement culture filtrates on the vascular systems of tomato cuttings*

Organism	Grade of browning in the dilution indicated[a]			
	0	1:4	1:8	1:16
Fusarium oxysporum f. *lycopersici* (R5-6)	4	4	3	2
Fusarium oxysporum f. *lycopersici* (A15-8)	4	3	2	2
Fusarium oxysporum f. *conglutinans*, race 1	4—	2+	2—	1
Fusarium oxysporum f. *pisi*, race 1	4	2+	2	1
Fusarium oxysporum f. *pisi*, race 2	4	2+	2	1
Fusarium oxysporum f. *vasinfectum*	4	4	3	2
Fusarium oxysporum from brome	4	2+	2	1
Fusarium solani f. *pisi*	4	3	2	1
Fusarium solani f. *phaseoli*	4	3	2	1
Alternaria solani	1[b]	0	0	0
Botrytis cinerea	0	0	0	0
1:4 Czapek's medium	0	0	0	0

[a] 0=no vascular browning; 1=slight browning at the base of the stem or in some leaf axils; 2=browning in the lower third of the stem; 3=browning in the lower ⅔ of the stem; 4=browning from the base to the apex of the stem.
[b] Only slight browning of the bundles in the leaf axils.

Next 5.0 ml. of 0.1N I_2 was added and the sample placed in the dark for 20 minutes. After acidifying with 4 ml. of 2M H_2SO_4 the residual I_2 liberated was titrated with 0.1N $Na_2S_2O_3$ using starch as an indicator. The m.e. of reducing groups liberated were determined from a standard curve prepared from data with glucose. The activity was expressed in units per ml. of preparation, a unit being the amount of enzyme required to liberate 0.05 milliequivalent reducing groups in 5 minutes in a 5-ml. aliquot from the reaction mixture.

EXPERIMENTAL RESULTS.—*Effects of culture filtrates on the vascular systems of tomato cuttings.*—Cuttings 12 to 20 in. tall of resistant and susceptible tomato plants were placed in replacement culture filtrates under both greenhouse and controlled conditions. The organisms used and the composite indices from 4 plants in each of 2 or more tests are presented in Table 1. In the replacement culture filtrates of strain R5-6, cuttings began to show wilt after 24 hours and were moderately to severely wilted after 2 days. When the epidermis and cortex were peeled back at this stage, the vascular bundles showed browning only in scattered spots, chiefly near the base of the stem. After 2 more days in water, cuttings which had been in the undiluted filtrate did not recover from wilt but those in the diluted filtrates recovered partially. There was some leaf blade injury, but the symptoms consisted primarily of the flagging of petioles and the upper stems followed by dark browning of the vessels. The browning was similar to that in naturally-infected plants, which had become severely diseased. There was very little softening of the base of the stem, but when plants were left in the filtrate for 4 days the portion of the stems submerged in the filtrate usually was very soft. Very little, if any, water or filtrate was taken up during the last 2 days in those plants which showed the greatest browning. The browning produced by filtrates from the mildly pathogenic strain (A15-8) was only slightly less than that produced by filtrates from the highly pathogenic strain (R5-6). In some tests it was impossible to distinguish any differences between the 2 strains. Filtrates from f. *vasinfectum* cultures produced as intense browning as did those from f. *lycopersici*. All of the remaining fusarial organisms produced replacement culture filtrates which induced almost as intense browning in the undiluted filtrates as did the replacement cultures from the cotton and tomato wilt organisms. However, the intensity of the browning at the 1:4, 1:8, and 1:16 dilutions was always slightly less than in corresponding dilutions of the cotton and tomato fusaria. Most of the browning in the 1:16 dilution was in the bundles of the lower portion of the stem and in the axils of the leaves. Little or no vascular browning entity was produced by the filtrates from the *Alternaria* and *Botrytis* cultures. It was not possible to distinguish any differences in the intensity of vascular browning in resistant and susceptible tomato cuttings.

When the filtrate of each organism was boiled gently for 10 minutes before use no vascular browning occurred except for a slight amount in the axils of the leaves when undiluted filtrate was used. Thus the vascular browning entity produced by each of the fusaria is a thermolabile entity as shown for the tomato fungus by Scheffer and Walker (7).

In addition to the production of the vascular browning, other types of injury were noted on tomato cuttings. In those treated with cotton filtrate water-soaked areas involving ½ or more of the leaf blades appeared in 24–48 hours after treatments were begun. The injured areas soon became dry and brittle. Injury to leaf blades by filtrates of other fusarial organisms consisted of scattered, round to oval, areas similar to but much less severe than those produced by the cotton organism filtrate. Severe leaf blade injury was produced by the *Alternaria* and *Botrytis* replacement culture filtrates. Folding of the stems similar to that described by Gäumann et al. (1) was induced by the undiluted filtrates of all fusarial cultures. The leaf-blade injury and stem folding was equally severe with heated and unheated filtrates.

To prepare filtrates from cultures of *Pseudomonas solanacearum* the bacteria were seeded heavily in 100 ml. of potato-dextrose broth and grown in shake culture for 24 hours. After centrifugation the supernatant was saved and the bacteria were collected and suspended in a 1:4 potato-dextrose broth medium for 24 hours on a shaker and filtered. The supernatant, the replacement filtrate, and noninoculated potato-dextrose broth control were filtered through a layer of asbestos and celite, and finally through a fine sintered glass filter to remove any bacterial cells present. Noninoculated broth and the supernatant were used at dilutions of 1:4, 1:8 and 1:16; the replacement filtrate was used undiluted and at 1:2 and 1:4 dilutions. When cuttings were treated with 1:4 dilution of potato dextrose broth slight browning was noted in the bundles at the leaf axils. In undiluted supernatant browning occurred which was less intense than in any of the undiluted *Fusarium* replacement filtrates. The intensity of browning decreased as dilution of the supernatant increased. At 1:4 dilution the browning extended from the base upward for about two-thirds of the length of the stem. At 1:8 dilution browning was confined to the base of the stem and to the bundles at the leaf axils. In 1:16 dilution browning occurred only at the leaf axils. Browning occurred with undiluted replacement culture about equal in intensity with the 1:8 dilution of the supernatant, while that obtained with 1:2 dilution was similar to that with 1:16 supernatant. No browning occurred with the 1:4 replacement filtrate. When noninoculated broth, the supernatant, and the replacement filtrate were used undiluted and at 1:4 dilution after being boiled for 10 minutes, browning occurred only at the leaf axils.

Effect of culture filtrates on the vascular systems of cotton cuttings.—In order to determine whether the vascular browning entity produced by fusaria would cause browning in cotton as well as in tomato, cuttings of resistant and susceptible cotton plants were treated with undiluted replacement culture filtrates, with 1:4 dilution, and with sterile replacement culture medium. Browning occurred with each filtrate. It was generally slightly less intense in cuttings treated with the 1:4 dilutions than those treated with the undiluted filtrates. None occurred in the noninoculated medium. The browning differed somewhat from that produced in tomato cuttings in that very little occurred in the woody stele in the lower hypocotyl while it was very intense in all bundles from slightly below the cotyledonary node up to the apex of the stem and into the petioles of the leaves.

Effect of bran culture filtrates on tomato and cotton cuttings.—Gothoskar et al (3) demonstrated that the production of the vascular browning entity was much greater in culture filtrates prepared from wheat bran cultures of the tomato Fusarium wilt fungus. Since no browning was obtained with replacement culture filtrates of *Alternaria solani* and *Botrytis cinerea*, filtrates of these 2 fungi and the R5-6 strain of the tomato fungus prepared from wheat bran cultures were tested on tomato cuttings. The fungi were grown for 5 days on 20 gm. of sterile Pillsbury's wheat bran, moistened with 20 ml. of water. Two hundred ml. of water were poured over the bran culture and allowed to stand overnight. The cultures were then filtered to remove the bran and the filtrates were either centrifuged or passed through a layer of asbestos and celite in a Büchner funnel. The filtrates in dilutions of 1:4, 1:8 and 1:16 were tested on tomato cuttings under greenhouse conditions. After 24 hours the leaf blades of tomato cuttings treated with the *Alternaria* filtrate were severely injured. Less leaf-blade injury was noted on plants treated with *Botrytis* filtrates. Only slight leaf-blade injury was noted on the cuttings treated with *Fusarium* filtrate. Pronounced vascular browning was obtained with the *Fusarium* filtrate up to the 1:16 dilution. In the cuttings treated with the *Alternaria* filtrates large sunken black areas developed extending from the epidermis into the pith and fine black streaks extended along the bundle beyond these areas. In cuttings treated with the *Botrytis* filtrates there was browning less intense but similar otherwise to that induced by the *Fusarium* culture and the cortical tissue at the base of the stem became softened. When bran filtrates of the several fusaria were applied to cotton cuttings the vascular browning was more intense than that secured with replacement culture filtrates.

Effects of culture filtrates on the vascular systems of pea and cabbage.—When cuttings of pea and cabbage were treated under greenhouse conditions with replacement filtrates from the vascular fusaria and from the pea-root-rot *Fusarium*, plants took up very little of the filtrates and no browning occurred. Control plants in water also took up very little water indicating that some type of plugging occurred which was not induced by the filtrate. To secure a higher concentration of the browning metabolite bran culture filtrates were then used. Pea varieties susceptible to wilt and near wilt, susceptible to near wilt and resistant to wilt, and resistant to both wilt and near wilt were used as test plants. Filtrates from *F. oxysporum* f. *pisi*, races 1 and 2, f. *conglutinans*, race 1, f. *lycopersici*, f. *vasinfectum*, and *F. solani* f. *pisi* in dilutions of 1:2, 1:4, 1:8 and 1:16 were applied twice daily for 2 days; the cuttings were then placed in water for 2 days. All 3 varieties showed vascular browning predominantly in the lower portion of the stem and occasionally in a bundle in the upper portion of the stem. Browning was intense in 1:2 and in 1:4 dilutions, less so in 1:8, and it was absent in 1:16. No difference in the intensity of browning was noted between the 3 varieties. No browning was observed in cuttings treated with filtrate from sterile bran.

Cuttings of resistant and susceptible cabbage plants with 6–8 expanded leaves were treated with 1:4 dilution of bran culture filtrates prepared from the same organisms which had been tested on pea. The filtrates of all the organisms induced browning in cuttings of

TABLE 2.—*Pectinmethylesterase and polygalacturonase activity of wheat bran and replacement culture filtrates*

Organism	Culture Filtrate	PME[a]	PG[b]	Ratio PME:PG
Botrytis cinerea	bran	1.912	1.73	1.1:1
	replacement	0	0.058	———
Alternaria solani	bran	0.074	0.008	9.2:1
	replacement	0	t[c]	———
F. o. f. *lycopersici* (R5-6)	bran	0.388	0.004	97.0:1
	replacement	0.073	t	———
F. o. f. *lycopersici* (A15-8)	bran	0.432	0.006	72.0:1
	replacement	0.136	t	———
F. o. f. *vasinfectum*	bran	0.377	0.004	94.3:1
	replacement	0.102	t	———
F. o. f. *conglutinans*, race 1	bran	0.297	0.015	19.8:1
	replacement	0.045	t	———
F. o. f. *pisi*, race 1	bran	0.547	0.013	42.1:1
	replacement	0.064	t	———
F. o. f. *pisi*, race 2	bran	0.487	0.011	44.3:1
	replacement	0.062	t	———
F. s. f. *pisi*	bran	0.273	0.004	68.3:1
	replacement	0.057	t	———
F. s. f. *phaseoli*	bran	0.386	0.004	96.5:1
	replacement	0.092	t	———
F. oxysporum	bran	0.408	0.017	24.0:1
	replacement	0.054	t	———
Pseudomonas solanacearum	Potato dextrose broth	3.014	0.095	31.7:1

[a] Mg. methoxyl removed per ml. filtrate.
[b] Units of polygalacturonase.
[c] 0.002 units of polygalacturonase or less.

both varieties. The discoloration did not occur in the woody stele of the lower stem but was intense in vascular bundles throughout the succulent portion of the stem and leaf axils. No browning was noted in the check plants treated with a filtrate prepared from sterile wheat bran. When heated bran filtrates were used on pea and cabbage cuttings, only very slight traces of browning were observed.

Enzyme assays.—Pectinmethylesterase (PME) and polygalacturonase (PG) assays were run on the filtered washings of 4- or 6-day-old wheat bran cultures and on 24-hour replacement culture filtrates of all the fungi listed in Table 1. In addition 4-day-old potato-dextrose broth shake cultures of *Pseudomonas solanacearum* were assayed. PME was assayed by measuring the mg. of methoxyl groups hydrolyzed from a 1 per cent pectin solution by the enzyme present in 1 ml. of culture filtrate in 3 hours. The PG activity was expressed in units per ml. of preparation, a unit being the amount of enzyme required to liberate 0.05 milliequivalent reducing groups in 5 minutes in a 5 ml. aliquot from a 100 ml. reaction mixture of 0.5 per cent sodium polypectate. Results are in Table 2.

All the fungi tested produced more PME and PG on bran than in replacement culture. This may have been due to the pectin present in the bran substrate. All fusaria and *P. solanacearum* produced large amounts of PME in relation to PG, while *B. cinerea* produced very little of either on that medium. Both of the two last mentioned organisms produced no measurable PME and very little PG in replacement culture.

DISCUSSION.—Scheffer and Walker (7) and Gothoskar et al (3) have proposed that wilting of tomato plants infected with *Fusarium oxysporum* f. *lycopersici* is brought about by the action of an enzyme, pectinmethylesterase, which acts upon the walls of the xylem elements and releases a viscous material into the vessels which reduces translocation and eventually causes wilt. The browning may be associated with phenolic substances, released from the cells, and their subsequent polymerization to melanins by the action of polyphenoloxidases present in the host cells. Gäumann (2) has recently described a method for the precipitation of a substance, vasinfuscarin, from crude culture filtrates of *Fusarium oxysporum* f. *lycopersici*, *Fusarium oxysporum* f. *vasinfectum*, and *Gibberella fujikuoroi* (Saw.) Wr., which was capable of browning the xylem vessels of tomato plants. No suggestion was made, however, as to the mode of action of this compound. It was suggested that vasinfuscarin might be an enzymatic protein. It may eventually be shown to contain pectinmethylesterase as its active agent since the methods used to purify both compounds are similar.

It seems evident that the various wilting organisms used in this study produce in culture a common vascular browning and plugging entity. Filtrates prepared from cultures of *F.o.*f. *lycopersici*, *F.o.*f. *conglutinans*, race 1, *F.o.*f. *vasinfectum* and *F.o.*f. *pisi* races 1 and 2 were capable of inciting vascular browning and plugging in cotton, tomato, cabbage and pea plants. The culture filtrates of each of these wilting organisms showed relatively high PME and low PG activity. When resistant or susceptible varieties of the respective hosts were treated with the fungal filtrates they reacted similarly. This indicates that plants of resistant varieties as well as those of species that are not natural hosts possess no mechanism which can

withstand the effect of this disease-inducing entity. Hence resistance is in no way explained on the basis of tolerance to the pectic enzymes. The establishment of the pathogen in its respective susceptible host plants is therefore due to factors other than PME activity.

It appears that the fusarial root-rot organisms as well as the wilt organisms produce quantities of PME and PG which would enable them to be incitants of wilt if they grew in the xylem elements. Hence it seems that the factors which limit these organisms as parasites largely to the cortical tissues, where they cause a dry rot, is not associated with PME and PG activity.

B. cinerea, a soft rotting organism produces large quantities of both enzymes. These large quantities of PME probably diffuse ahead of the mycelium and split the methoxyl groups from the pectin molecule. This in turn provides a substrate for the PG which then splits the pectate or pectic acid into galacturonic acid. The middle lamellae are then dissolved and the disrupted cells provide suitable substrate for the mycelium. Protopectinase may also be important in soft rot; however, the existence of this enzyme has never been satisfactorily demonstrated.

The high production of PME by *Pseudomonas solanacearum*, as well as the fact that the bacteria become quickly transported throughout the vessels in which the organism is present, may be important in causing plants infected with this bacterium to succumb more rapidly than plants infected with *Fusarium*. Not only did this organism produce a very large quantity of PME but it also produced more PG than the fusaria. PG may be important in the breakdown of pits between xylem vessels and adjacent xylem parenchyma, and may account for the rapid spread of the organism from vessel to vessel as well as spread into the xylem parenchyma.

Since *A. solani* produced negligible amounts of PME and PG in bran and in replacement culture it is probable that the damage caused by filtrates of this organism to leaf blades and to stem pith was brought about by toxic byproducts such as alternaric acid which has been described by Pound and Stahmann (6) as a factor in the production of symptoms of early blight of tomato.

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LITERATURE CITED

1. Gäumann, E., S. Naef-Roth, and H. Kobel. 1952. Über Fusarinsäure, ein zweites Welketoxin des Fusarium lycopersici Sacc. Phytopath. Ztschr. **20**: 1-38.
2. Gäumann, E., C. Stoll, and H. Kern. 1953. Über Vasinfuscarin, ein drittes Welketoxin des Fusarium lycopersici Sacc. Phytopath. Ztschr. **20**: 345-347.
3. Gothoskar, S. S., R. P. Scheffer, J. C. Walker, and M. A. Stahmann. 1953. The role of pectic enzymes in Fusarium wilt of tomato. Phytopathology **43**: 535-536.
4. Hoagland, D. R., and W. C. Snyder. 1933. Nutrition of strawberry plant under controlled conditions: (A) Effects of deficiencies of boron and certain other elements; (B) Susceptibility to injury from sodium salts. Proc. Amer. Soc. Hort. Sci. **30**: 288-294.
5. Jansen, E. F., and L. R. MacDonnell. 1945. Influence of methoxyl content of pectic substances on the action of polygalacturonase. Arch. Biochem. **8**: 97-112.
6. Pound, G. S., and M. A. Stahmann. 1951. The production of a toxic material by Alternaria solani and its relation to the early blight disease of tomato. Phytopathology **41**: 1104-1114.
7. Scheffer, R. P., and J. C. Walker. 1953. The physiology of Fusarium wilt of tomato. Phytopathology **43**: 116-125.
8. Winstead, N. N., and J. C. Walker. 1953. Production of vascular browning by metabolites from several pathogens. (Abs.) Phytopathology **43**: 490.

TOXIC METABOLITES OF THE PATHOGEN IN RELATION TO FUSARIUM RESISTANCE [1]

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SUMMARY

Fusarium oxysporum f. *conglutinans* races 1 and 2 and *Fusarium oxysporum* f. *vasinfectum* were found to produce in culture metabolites specific in their effect on varieties within plant species which were susceptible, respectively, to these 3 organisms. Replacement culture filtrates in various dilutions were poured daily over the roots of susceptible and resistant plants of cabbage, radish and cotton, respectively, growing in quartz sand watered with Hoagland's nutrient solution.

Susceptible cabbage plants having 4 or more expanded leaves when treatments were begun showed within 48 hours veinclearing and yellowing of the cotyledons and lower leaves which suggested the earliest symptoms of yellows. Resistant plants with similar treatment remained free of these effects.

When replacement filtrates were prepared from either the cabbage or radish fusaria, susceptible radish plants showed external symptoms similar to those of infected plants. These filtrates had no effect on resistant radish plants. Cabbage plants resistant or susceptible to yellows remained free from effect when treated with filtrates prepared from the radish wilt organism. Susceptible cotton plants treated with filtrates prepared from mycelial mats of *Fusarium oxysporum* f. *vasinfectum* also showed external symptoms similar to those of infected cotton plants whereas resistant plants remained unaffected. When filtrates prepared from the cotton, cabbage, radish, tomato and pea Fusarium wilt organisms were tested on cotton, cabbage, and radish and when filtrates prepared from the cotton, cabbage, and radish organisms were tested on pea and tomato plants, the effects were always specific for the susceptible varieties of the host suscept of the organism concerned.

The metabolites produced by the cabbage yellows, radish wilt, and cotton wilt organisms which caused specific effects on susceptible varieties of the host caused no vascular browning. The metabolites were dialyzable, thermostabile, not affected by concentration or changes of pH from 1 to 12, and were not precipitated by ethyl alcohol.

It is suggested that *Fusarium oxysporum* f. *conglutinans* races 1 and 2 and *Fusarium oxysporum* f. *vasinfectum* produce metabolites of low molecular weight which have a specific effect on susceptible varieties and may in some way aid in the establishment of infection by the pathogen, in such varieties.

Scheffer and Walker (4) and Gothoskar et al (2) have shown that certain thermolabile metabolites of the tomato wilt organism (*Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder and Hansen), when taken up through the cut ends of tomato stems, induce plugging and wilt followed by vascular browning. Winstead and Walker (9) showed that a similar effect on cabbage and cotton stems with cut ends is induced by a water extract from bran cultures of the cabbage yellows fungus (*F. oxysporum* f. *conglutinans* (Wr.) Snyder and Hansen race 1), and the cotton wilt fungus (*F. oxysporum* f. *vasinfectum* (Atk.) Snyder and Hansen). They (9) also showed that the metabolites from the cabbage, cotton and tomato fungi are equally effective in inducing vascular browning, yellowing, and wilt on cabbage varieties resistant and susceptible to yellows and in producing plugging, wilting and vascular browning on tomato and cotton varieties resistant and susceptible to Fusarium wilt. In other words, the thermolabile metabolites have no relation to the specialization of f. *lycopersici* to susceptible varieties of tomato, of f. *conglutinans*, race 1, to susceptible varieties of cabbage, and of *f. vasinfectum* to susceptible varieties of cotton when taken up through cut ends. Pound and Fowler (3) have demonstrated that the radish (*Raphanus sativus* L.) wilt organism, *Fusarium oxysporum* f. *conglutinans*, race 2, and the cabbage yellows organism, f. *conglutinans*, race 1, incite identical diseases on radish varieties susceptible to wilt. Radish lines resistant to race 2 are also resistant to race 1. On the other hand, varieties of cabbage both susceptible and resistant to the yellows organism are resistant to the radish-wilt organism. The present investigation was directed toward a search for metabolites produced by *F. oxysporum* f. *conglutinans*, races 1 and 2, and f. *vasinfectum* which have a close relation to the specific pathogenicity of the organisms to susceptible varieties of cabbage, radish, and cotton, respectively. A preliminary report has been published (8).

METHODS AND MATERIALS.—For the major portion of the study on cabbage the resistant plants used were of the variety Jersey Queen which is homozygous for the gene pair, *RR*, controlling resistance to yellows (6, 7). The susceptible plants were of the variety Copenhagen Market which had been shown in previous tests to consist of close to 100 per cent of individuals homozygous for the gene pair, *rr*, controlling susceptibility to yellows. Radish variety Early Scarlet Globe (susceptible) and resistant line 39-52 as well as the cabbage varieties Jersey Queen and Copenhagen Market were used in the studies on the radish organism. The cotton varieties Coker 100 (resistant) and Stoneville (susceptible) were used in the studies on metabolites produced by *F. oxysporum* f. *vasinfectum*. When other resistant and susceptible varie-

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ties of cabbage and of other species were used, they will be indicated in connection with discussion of the experiment concerned.

Culture filtrates and replacement culture filtrates were prepared in the manner described elsewhere (9). A "culture filtrate" is that secured from a culture grown for 1–2 weeks on a modified Richard's solution and used in undiluted form or after various dilutions with water. A "replacement culture filtrate" is that secured when a mat of fungus from a culture on this medium was filtered off, washed, and placed in 1:4 dilution of Czapek's medium or in water, as indicated in the case at hand, for 24 or 48 hr. after which the fungus was removed and the filtrate used. The pH of these filtrates usually fell within the range of pH 6.0–pH 6.5.

Unless otherwise indicated, plants were grown in quartz sand which was watered daily with Hoagland's basal solution as described elsewhere (1).

EXPERIMENTAL RESULTS.—*Cabbage yellows.*—Since many experiments with various modifications were run, upon which final deductions were based, they will be described only in such detail as is necessary to indicate the results secured. Inasmuch as a previous work (9) showed that the enzymes which induced vascular browning were thermolabile and non-dialyzable, and not specific to susceptible varieties of cabbage, the first approach was to explore for toxic metabolites of lower molecular weight which might be absorbed through the roots of cabbage plants rather than through cut ends of stems.

The first experiment was with resistant and susceptible plants which had about 6 expanded leaves and which were grown in 4-in. clay pots. Culture filtrates were prepared and ½ the amount was autoclaved at 15 lb. pressure for 15 minutes. The heated and unheated filtrates were applied at various dilutions by pouring about 50 ml. over the surface of the sand of each pot, allowing any excess to drain out at the base of the container. In some cases only 1 treatment was made, in others it was repeated for as many as 5 consecutive days. It was soon obvious that the effects of heated and unheated filtrates were similar.

The effects upon plants varied in degree with variety and with the dilution and frequency of application of the filtrate. Symptoms produced were distinct from those which appeared on cuttings inserted in unheated extracts from bran cultures where vascular browning and plugging were the chief features (9). The effects in the current experiment were veinclearing, blackening adjacent to the main veins, yellowing of cotyledons and lower leaves, and sometimes but not always wilting of the entire plant. This wilting was not caused by plugging of the vessels. All the vessels of both treated and untreated plants became red within 2 hours when the eosin technique described by Scheffer and Walker (4) was used. These symptoms developed very promptly. The veinclearing and yellowing suggested, but were not identical with, the veinclearing of cotyledons and young leaves which are the very first signs of the disease when susceptible seedlings in the first-leaf stage are transplanted to infested soil and kept at a soil temperature favorable to rapid disease development (5). Some days later roots of treated plants turned dark and tended to soften while about this time or slightly later a mottle chlorosis appeared and increased in intensity on the leaves. The latter symptom was suggestive of mineral deficiency and was later shown to be so, since after treatment with filtrates ceased surviving plants recovered normal growth when Hoagland's solution was applied regularly. A comparison is shown in Fig. 1,A of the veinclearing in leaves of naturally infected plants with that of a treated plant.

With this general syndrome of symptoms in mind the effect of dilution of filtrate and of host variety may be considered. When undiluted filtrate was used, both resistant and susceptible plants were injured beyond recovery within 24 hours. As dilution of the filtrate increased, symptoms at any given level were relatively more severe on susceptible than on resistant plants; when a 1:16 dilution of filtrate was applied for 5 successive days, slight veinclearing appeared on 1 or more leaves of susceptible plants, but no signs appeared on resistant plants. It was thus evident that when the diluted heated filtrate was poured over the sand substrate a toxic metabolite was absorbed by the plant which induced veinclearing and other symptoms described. At high concentration this metabolite was not specific to susceptible plants but when it was diluted bleaching of interveinal tissue and root injury followed by mineral deficiency symptoms appeared only on susceptible plants.

This experiment was repeated several times with culture filtrate and with replacement culture filtrate and with some minor modifications as to dilution and times of application. Since the results, in general, were confirmatory the individual experiments are not reported in detail. The greatest difference in response of resistant and susceptible plants was attained when replacement filtrates were secured by placing mycelial mats from 1-week-old heavily seeded cultures in a 1:4 dilution of Czapek's medium for 24 hours. When the original mycelial mat was taken from 4-week-old cultures the replacement culture filtrate was only weakly toxic. When the mycelial mat from a 1-week-old culture was left in the replacement medium for 48 hours the filtrate was less toxic than from the 24-hour replacement culture. When water was used as the replacement medium the filtrate was less toxic than when 1:4 Czapek's medium was used.

Young resistant and susceptible seedlings in the first-leaf stage were transplanted to sand and to soil in stainless steel trays. The sand trays were watered with Hoagland's solution and the soil watered with tap water. After recovery of the plants from transplanting, the trays were inserted in Wisconsin soil temperature tanks and maintained at 20° C. Replacement culture filtrate was added on alternate days for a total of 6 treatments. No effects were observed at

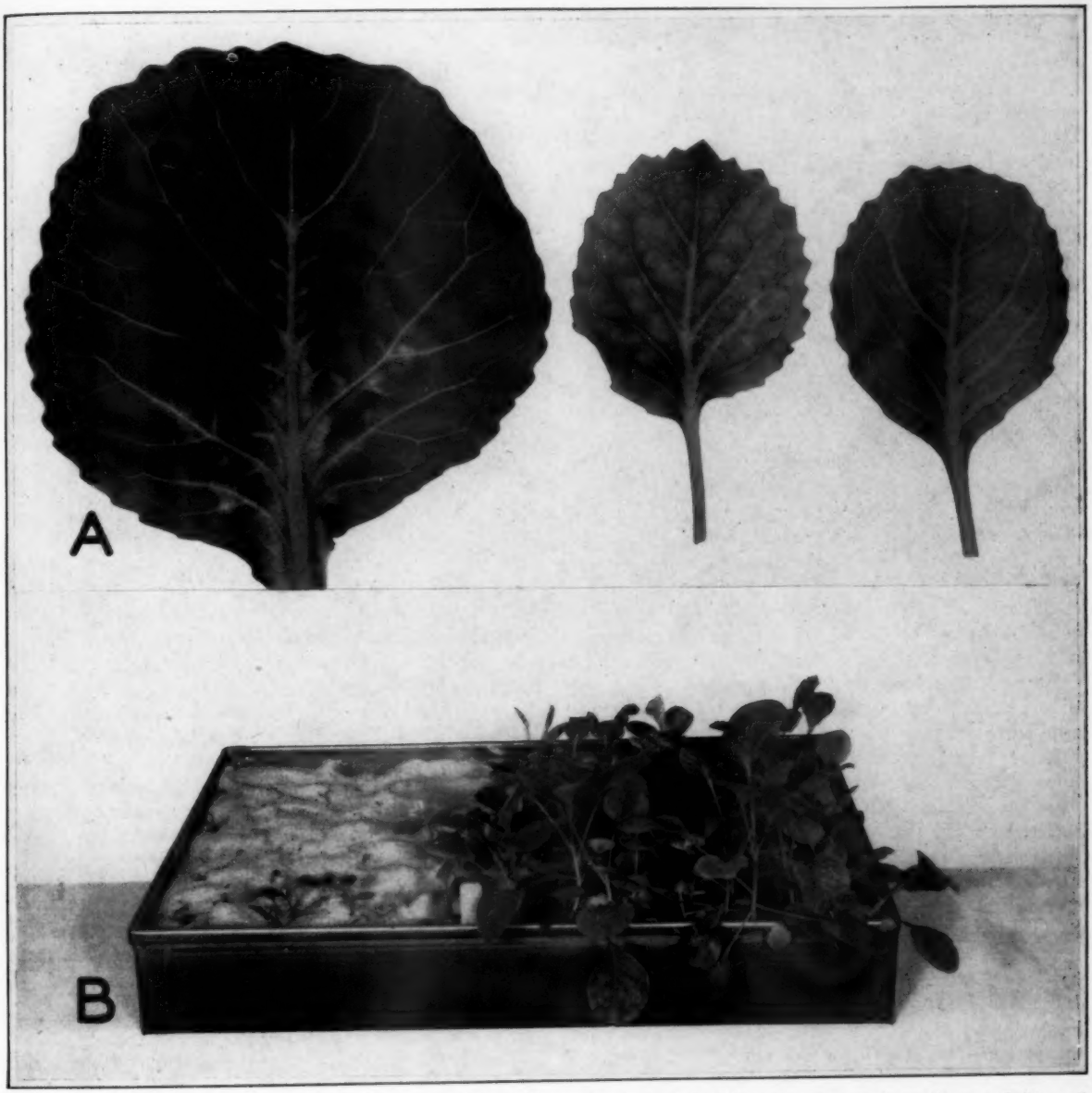

FIG. 1—A) Veinclearing in the leaf of a susceptible cabbage plant treated with replacement culture filtrate (left) and in leaves of a plant infected with *Fusarium oxysporum* f. *conglutinans* (center and right). B) Susceptible Copenhagen Market plants (left) and resistant Jersey Queen plants (right) treated with replacement culture filtrates for 7 days then watered with Hoagland's nutrient solution for 2 weeks.

any time on plants growing in soil. In susceptible plants growing in sand the cotyledons became yellow, showed some vein bleaching and some necrosis; the apical buds were killed and most of the plants died. Resistant plants growing in sand showed slight yellowing and deficiency symptoms, but apical buds were seldom injured. At the end of the treatments the sand was flooded with water which was siphoned off to dilute the toxic metabolites, and the sand was watered daily with Hoagland's solution. The susceptible plants had been killed or were near death and no recovery was observed. Except for an occasional plant in the resistant group all plants recovered from deficiency symptoms and resumed normal growth. This experiment was repeated several times with similar results. When the filtrate was fortified with salts to bring it up to the composition of the Hoagland's solution the same syndrome of disease and deficiency symptoms appeared. It was only after treatment with the filtrate ceased and the sand washed that surviving plants returned to normal growth in response to Hoagland's solution. In Fig. 1,B are susceptible (left) and resistant (right) plants treated for 7 days, the sand rinsed, and watering with Hoagland's solution

continued for 14 days. A few chlorotic susceptible plants survive while most resistant plants are growing normally.

The tests were extended to other resistant and susceptible varieties of cabbage. Copenhagen Market, Danish Ballhead, Oakview Ballhead, and Ferry's Hollander were included as susceptible varieties. Jersey Queen, Wisconsin Ballhead, Wisconsin Copenhagen, Badger Market, and Wisconsin Golden Acre were single-gene resistant varieties and Wisconsin Hollander and Bugner were multigenic resistant. All susceptible varieties responded in the same manner as Copenhagen Market, while all resistant varieties were similar in reaction to Jersey Queen. This showed that the response to the toxic metabolites in the heated replacement cultures was definitely correlated with resistance and susceptibility to infection by *Fusarium oxysporum* f. *conglutinans*, race 1.

The effects of culture filtrates and replacement culture filtrate on plants known not to be hosts of the cabbage yellows organism were studied next. Varieties of tomato, cotton, and pea susceptible and resistant to Fusarium wilt diseases of the respective species were grown in sand and treated with various dilutions of the filtrate. Undiluted filtrate or dilutions up to 1:10 caused death of all plants. When the filtrate was diluted to 1:16 and 1:25 no effects were noted. Undiluted filtrates from cultures of the tomato wilt, pea wilt, pea near-wilt, and cotton wilt organisms when applied to cabbage killed all plants while dilutions of 1:12 or greater had no effect. Cabbage filtrate diluted 1:16 caused the usual effect on susceptible cabbage but resistant plants showed only slight injury and recovered when Hoagland's solution was applied after treatment. These results indicate that in the undiluted filtrates from any of the wilt organisms nonspecific metabolites occur which cause severe injury on all plants tested. On the other hand at concentration levels at which injury is confined to susceptible cabbage no injury occurs on tomato, pea, and cotton. Likewise filtrates from cultures of the pathogens of tomato, pea, and cotton when used at the same concentration level are injurious neither to susceptible nor to resistant cabbage. Likewise replacement culture filtrates from the pea wilt, pea near wilt, tomato wilt, and cotton wilt organisms had no effect on either resistant or susceptible cabbage. Replacement filtrates of the cabbage yellows organism also had no effect on plants resistant and susceptible to these 4 wilt diseases. Hence a thermostabile metabolite was present only in culture and replacement filtrates of the cabbage yellows organism which incites specific effects only on susceptible cabbage plants.

Radish wilt.—Pound and Fowler (3) have demonstrated that the cabbage yellows organism, *Fusarium oxysporum* f. *conglutinans*, race 1, incites radish wilt in susceptible varieties of radish, while the radish wilt organism, *Fusarium oxysporum* f. *conglutinans*, race 2, does not incite yellows in varieties of cabbage susceptible to the cabbage yellows organism nor wilt resistant radish lines. A study was made of the effects of replacement culture filtrates prepared from the radish wilt and cabbage yellows incitants on cabbage and radish. Plants of the cabbage varieties Jersey Queen and Copenhagen Market (resistant and susceptible respectively to yellows) and of the radish variety Early Scarlet Globe and breeding line 39-52, (respectively, susceptible and resistant to wilt) having 6–14 leaves were tested. Replacement culture filtrates were poured at daily intervals over the surface of the sand in which plants were growing.

When 24-hour replacement culture filtrates prepared from 2-week-old mycelial mats of the radish wilt and cabbage yellows organisms were tested on susceptible radish plants, the cotyledonary and lower leaves wilted after 2 or 3 days of treatment. This wilting like that described for cabbage in the previous section was not caused by plugging of the vessels. Yellowing usually appeared first near the midvein at the tip of the leaf and spread rapidly towards the leaf base. Within 48 hours after the first yellow area appeared the entire leaf became yellow and then either dehisced or dried up. Within 8–10 days this wilting and chlorosis usually involved the whole plant. These effects on treated plants were quite similar to the external symptom syndrome of infected plants except for the more rapid appearance of symptoms. If treatments were stopped and Hoagland's solution added at any time before the youngest leaves and apical bud became affected, the treated plants recovered except for those leaves which had become chlorotic. Plants treated with replacement culture filtrates that had been autoclaved for 15 minutes at 15 lb. pressure or with replacement filtrate brought up to the nutrient level of Hoagland's solution reacted similarly.

Many roots of severely affected plants were discolored. When the plants were examined further for vascular browning none was observed in either the taproot or hypocotyl. However, vascular traces leading to dead or dehisced leaves were always browned adjacent to the abscission scar. Similar effects were noted on plants having from 6 to 14 expanded leaves treated with a 1:2 dilution of the replacement culture filtrate prepared from either the cabbage yellows or radish wilt organisms. The effect on Early Scarlet Globe plants treated with a 1:2 dilution of replacement filtrate for 4 days is pictured in Fig. 2,A.

When a 1:2 dilution of the radish and cabbage filtrates, brought up to the nutrient level of Hoagland's solution, were added to resistant radish no effects were observed within 6 days of treatment (Fig. 2,B). However, if undiluted replacement filtrate was used the lower leaves usually showed some wilt and slight chlorosis within 4–5 days. The symptoms appeared and developed much more slowly in these resistant plants than in susceptible plants. Identical results were obtained with replacement filtrates from both the cabbage and radish organisms.

When mycelial mats were left suspended in the replacement medium for 48 hours, the undiluted fil-

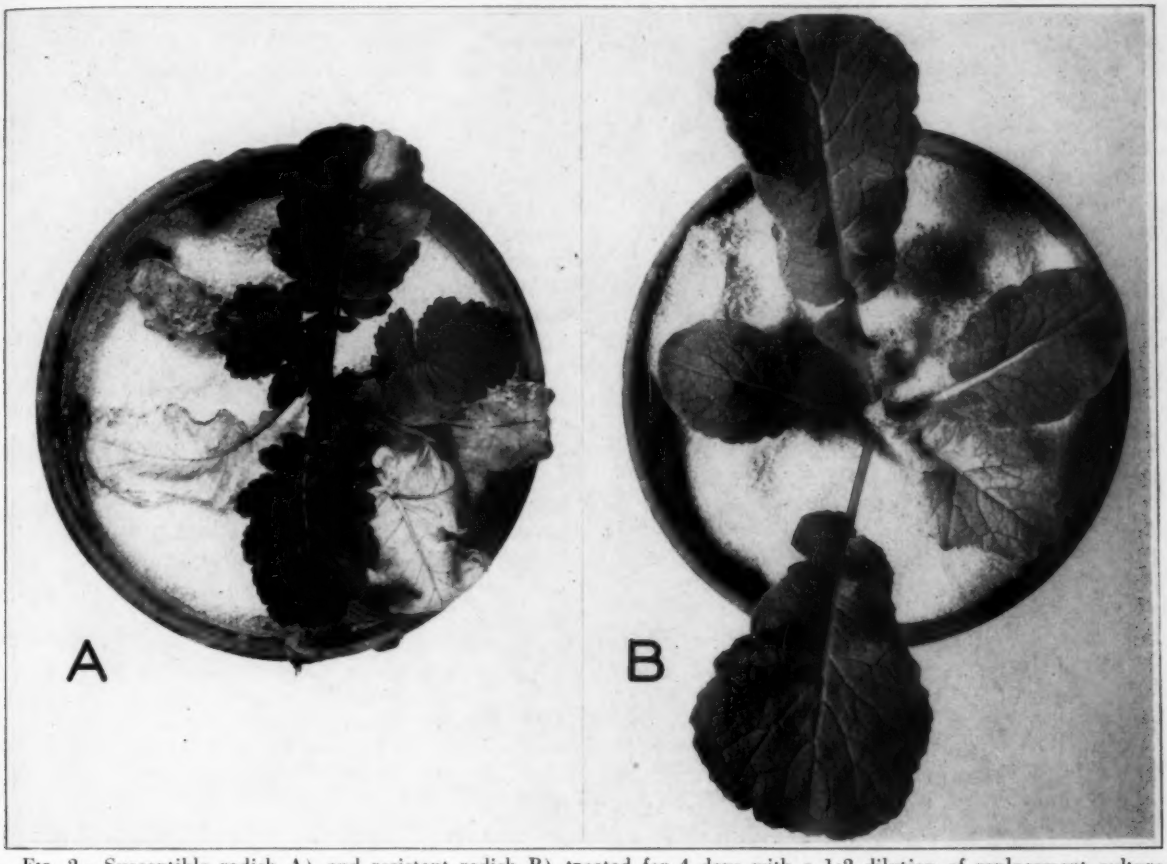

FIG. 2.—Susceptible radish A) and resistant radish B) treated for 4 days with a 1:2 dilution of replacement culture filtrate prepared from *Fusarium oxysporum* f. *conglutinans* race 2.

trates of both organisms induced only slight effect on plants of the Early Scarlet Globe variety. Young mycelial mats obtained from heavily seeded 1-week-old cultures or 2-week-old cultures produced this specific metabolite more abundantly than 4-week-old mycelial mats which showed signs of autolysis.

Replacement culture filtrates of the radish wilt organism were tested on plants known not to be hosts of the radish organism. Varieties of cabbage, tomato, cotton and pea resistant and susceptible to *Fusarium* wilt diseases were treated with undiluted and 1:2 dilutions of the replacement filtrate prepared from the radish organism. When undiluted filtrates were tested on cabbage the effects were quite similar to those produced in resistant radish. That is, some slight wilting and chlorosis were evident within 5–6 days. However, no consistent difference in the effect on varieties of cabbage resistant and susceptible to yellows was noted. No effect was observed when 1:2 dilutions were used. Replacement filtrates prepared from the radish wilt organism had no effect on the tomato, cotton, and pea plants, nor did filtrates prepared from the fusaria which infect these hosts have any effect on radish plants.

Cotton wilt.—Since a toxic entity specific in its effect on cabbage and radish was found in the replacement culture filtrates of the cabbage yellows and radish wilt organisms, respectively, a study was made on the effects of replacement culture filtrates prepared from *Fusarium oxysporum* f. *vasinfectum* (Atk.) Snyder & Hansen. These filtrates were tested on Coker 100 resistant and Stoneville susceptible plants. The replacement culture filtrates were prepared in the manner previously described. They were poured at daily intervals over the sand substrate in which plants of various sizes were growing.

When the replacement culture filtrate was added to plants having 4–6 expanded leaves the cotyledonary leaves of susceptible plants turned yellow on the fourth or fifth day of treatment. After 6 days of treatment the cotyledonary leaves dehisced and 1 or more leaves gradually became chlorotic and wilted. None of the resistant plants showed any effect. The plants were then divided into 2 lots. To half of the plants Hoagland's solution was added. To the other half filtrate was added for 2 more days. On the susceptible plants to which the filtrate was added chlorosis and wilting of leaves increased and the latter finally dehisced. The aerial symptoms produced by the toxic filtrate on susceptible plants were very similar to those of cotton plants which had been inoculated 4 weeks earlier. The susceptible plants treated with filtrate for 6 days

resumed normal growth when the plants were watered with Hoagland's solution except for the dehiscence of the yellowed cotyledons and of the leaves which had shown severe wilt. Some plants treated for 8 days did not recover. Plants having from 2–12 expanded leaves reacted similarly to the filtrate except that the smaller plants wilted earlier than did the larger plants. When plants having only cotyledonary leaves were treated for 6 consecutive days no toxic effect was observed.

Almost all the roots of treated susceptible plants were discolored and many were killed. When the plants were examined further by peeling the cortex, no vascular browning was observed in either the woody stele or in the bundles of the upper stem of plants treated for 6 days. No vascular browning was found in plants which showed severe wilt after treatment for 8 days, but browned bundles were prevalent in the axils of leaves which had dehisced. When the eosin technique (4) was used, no vascular plugging was evident in these wilted plants. After cessation of treatment and application of Hoagland's solution daily, healthy secondary roots developed along the upper tap root and occasionally from discolored secondary roots.

On resistant plants no effect of the filtrate was evident on aerial portions of resistant plants after 8 days' treatment. The roots of such plants showed little or no injury. The effects of 6 days' treatment on the aerial and on subterranean portions of resistant and susceptible plants, compared with an untreated plant are shown in Fig. 3.

When filtrates were prepared from mycelial mats from 4-week-old cultures no injury resulted on susceptible plants after 6 days of treatment. Thus the specific toxic metabolite of the cotton fungus like that of the cabbage and radish fungi was produced most abundantly by young mycelial mats which showed no signs of autolysis. Other techniques were used in attempts to increase the concentration of the specific toxic entity. When mycelial mats were left suspended in the replacement culture fluids for 48 hours, no toxic effect was produced on susceptible plants. If replacement cultures were placed on a mechanical shaker for 24 hours no injury was observed on susceptible plants after 8 days' treatment. Mycelial mats were suspended in water, standard replacement culture medium, replacement medium with double sugar content, and replacement medium with double mineral content. No effect was observed on plants treated with the water filtrate. The 24-hour replacement culture filtrates from the standard replacement medium and the double mineral medium showed an effect similar to that previously described. The filtrates prepared from the double sugar medium, however, produced slight yellowing of the cotyledons and wilting of the susceptible plants 72 hours after the first treatment, and an occasional resistant plant showed slight wilt at 4 days. After 5 days of treatment all the susceptible plants were severely wilted with a 1:2 dilution of this filtrate, while after 6 days of treatment an occasional resistant plant showed slight wilt. Only susceptible plants were affected by a 1:4 dilution of the high sugar filtrate. Thus sugar content of the replacement culture filtrate has a definite influence on production of the specific toxic metabolite of the cotton fungus, a fact not observed with the cabbage fungus.

Replacement culture filtrates were prepared from the cabbage yellows, pea wilt, near wilt, and tomato wilt organisms. No effect was noted on either susceptible or resistant cotton plants treated for 10 days. Replacement culture filtrates prepared from the cotton fungus showed no effect when applied to resistant and susceptible plants of cabbage, pea and tomato growing in sand substrate.

Properties of the toxic thermostabile metabolites.—Early in these investigations the possibility that the selective toxic entities found in the replacement culture filtrates of the cabbage, radish, and cotton fungi were identical or similar to the vascular plugging and browning entities of Scheffer and Walker (4) was eliminated. The vascular browning entity appears to be the enzyme, pectinmethylesterase (2). Its activity is not lost when dialyzed for 24 hours. No effect was noted on susceptible plants when dialyzed preparations of the cabbage yellows and radish wilt organisms were added for 10 days or of the cotton wilt organism for 15 days. When the filtrates of these organisms were dialyzed in the refrigerator against an equal quantity of distilled water the toxic entities passed through the membrane and were shown to be present in the water outside the membrane. Hence, it was evident that these toxic entities were not closely related to the vascular plugging entity.

In many of the experiments heated as well as unheated filtrates were used; identical results were always obtained. Replacement culture filtrates prepared from the cabbage, radish and cotton organisms also retained their specific toxicity when autoclaved for 2 hours at 15 lb. pressure. In addition, attempts to isolate the fungi from plants treated with the heated and unheated filtrate failed. Hence, these specific effects are produced by thermostabile toxic entities produced in culture by these 3 organisms. No reduction in the toxicity of the filtrates to their respective host was observed when they were passed through Seitz or fine sintered glass filters.

The active components of the 3 organisms present in replacement filtrates and in concentrated preparations (3 l. filtrate reduced to 150 ml. by distillation in vacuum) were not inactivated by extreme changes in pH. The replacement filtrate had a pH of approximately 6.3. When the filtrate was reduced to pH 1.0 or raised to pH 12.0 no loss of activity was noted when the filtrate was readjusted to pH 6.3. However, when the filtrates were made alkaline a white precipitate formed. When the supernatant was tested for activity on growing plants, little or no loss was noted. When 150 ml. of the concentrated filtrate was added to 1 l. of ethyl alcohol, a tan precipitate was formed. This precipi-

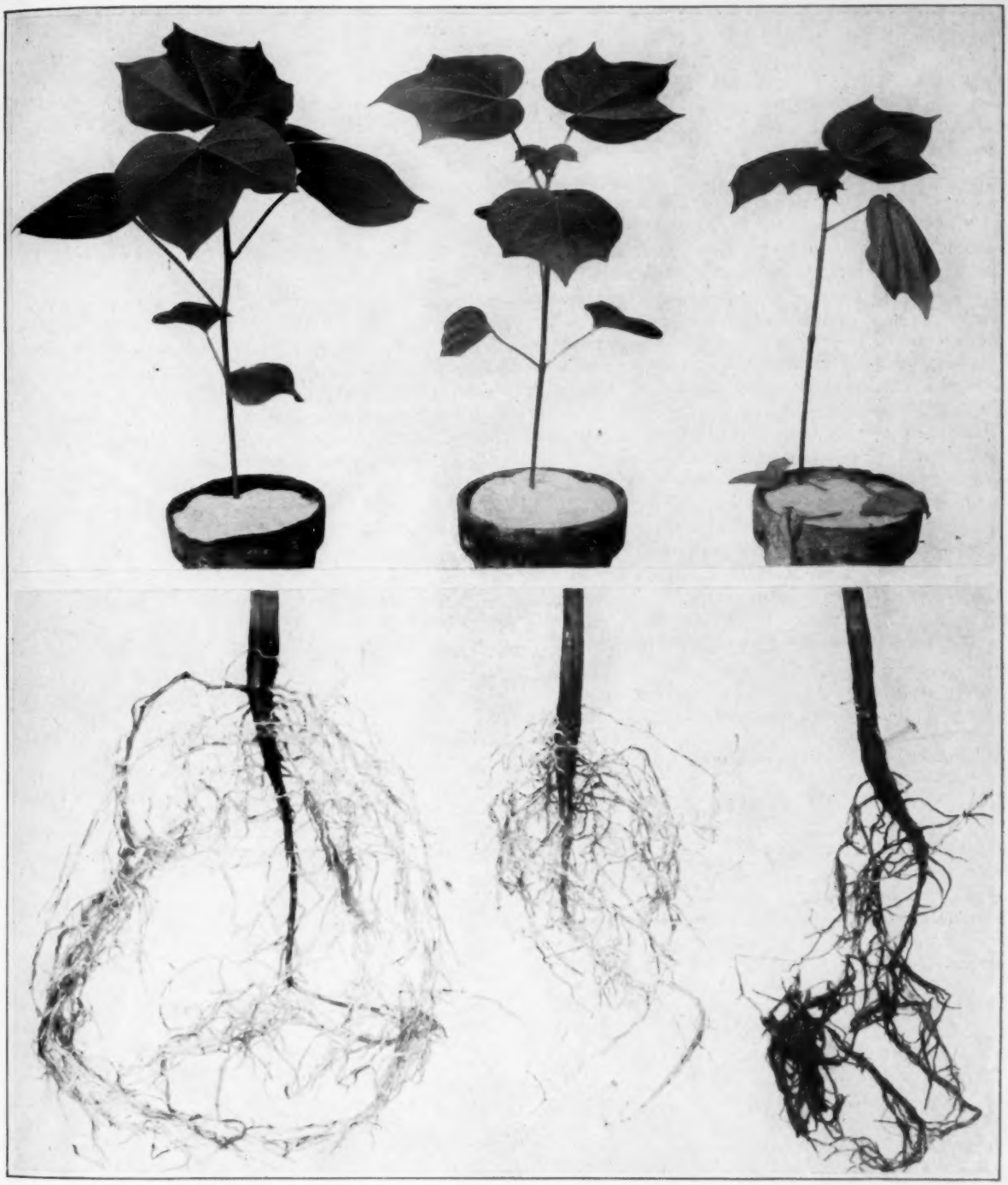

FIG. 3.—Effect of replacement culture filtrates of *Fusarium oxysporum* f. *vasinfectum* on tops and roots of susceptible Stoneville (right) and resistant Coker 100 (center) cotton plants. An untreated Stoneville plant is shown on the left.

tate contained no demonstrable quantity of the active principles while the supernatant after the removal of the alcohol showed only a slight loss of activity.

DISCUSSION. — Pectinmethylesterase (PME) probably plays a very important role in vascular plugging and browning as has been demonstrated by Gothoskar et al (2) for *Fusarium oxysporum* f. *lycopersici* and for several other wilting organisms by Winstead and Walker (9). However, plants resistant or susceptible to the various wilt diseases react similarly to this enzyme. Hence PME has no relation to varietal resistance within a given host species to a particular

form of *Fusarium*, and none to the specific pathogenicity of a form of *Fusarium* for one particular host species. The metabolites produced in culture by *Fusarium oxysporum* f. *conglutinans* races 1 and 2 and *Fusarium oxysporum* f. *vasinfectum* when applied at specified dilutions affect only plant species, and varieties within a species, which are susceptible to the particular pathogen.

These 3 fusaria each produced metabolites in culture which when taken up through the roots by the respective susceptible hosts incite symptoms suggestive of the early stages of the disease in naturally infected plants. However, the most striking effect is the very close relation which they have to the specific pathogenicity of the organism to susceptible varieties of their host species. The role that these metabolites may play in either the establishment of infection or in the progress of the disease within plants is unknown. However, due to the specificity of each on a particular host suscept, it might be suggested that these metabolites play a role in the establishment of infection.

These metabolites all appear to be quite similar in their effects, except for the hosts affected, and in their properties. They appear to be completely unrelated to pectinmethylesterase, the vascular plugging enzyme. These 3 metabolites all appear to be low molecular weight compounds which are quite stable at extremes of pH and at high temperatures.

Replacement filtrates from the cabbage and radish organisms both caused identical effects on susceptible radish plants, indicating that they both produce either an identical metabolite or metabolites which may differ but which produce identical effects on susceptible radish plants. At the same time the cabbage fungus produced in culture a metabolite which was specific in its effect on cabbage whereas the radish filtrate did not contain this metabolite in demonstrable quantities. This suggests that the cabbage yellows organism either produces 2 metabolites, one having a specific effect on susceptible cabbage and the other on radish, or 1 metabolite which has a selective effect on susceptible varieties of cabbage and radish. Since these 2 organisms have many common hosts and have been considered by Pound and Fowler (2) to be distinct races of the same form of *Fusarium oxysporum*, it might be suggested that the radish wilt organism has lost either the ability to produce the part of the common metabolite necessary for the infection of cabbage plants, or more likely the ability to produce the specific cabbage metabolite.

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LITERATURE CITED

1. Gallegly, M. E., Jr., and J. C. Walker. 1949. Plant nutrition in relation to disease development. V. Bacterial wilt of tomato. Amer. Jour. Bot. **36**: 613-623.
2. Gothoskar, S. S., R. P. Scheffer, J. C. Walker, and M. A. Stahmann. 1953. The role of pectic enzymes in Fusarium wilt of tomato. Phytopathology **43**: 535-536.
3. Pound, G. S., and D. L. Fowler. 1953. Fusarium wilt of radish in Wisconsin. Phytopathology **43**: 277-280.
4. Scheffer, R. P., and J. C. Walker. 1953. The physiology of Fusarium wilt of tomato. Phytopathology **43**: 116-125.
5. Walker, J. C., and Rose Smith. 1930. Effect of environmental factors upon the resistance of cabbage to yellows. Jour. Agr. Res. [U.S.] **41**: 1-15.
6. Walker, J. C. 1933. Yellows-resistant lines of Jersey Wakefield cabbage. Jour. Agr. Res. [U.S.] **46**: 639-648.
7. Walker, J. C. 1933. The Jersey Queen cabbage. Rept. Md. Agr. Soc. **17**: (1932) 328-330.
8. Winstead, N. N., and J. C. Walker. 1953. Metabolites of vascular fusaria of cabbage and cotton in relation to resistance. (Abs.) Phytopathology **43**: 489-490.
9. Winstead, N. N., and J. C. Walker. 1954. Production of vascular browning by metabolites from several pathogens. Phytopathology **44**: 153-158.